Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

Potencial Tecnológico y Digestibilidad *ín vítro* de coproductos del dátil ilicitano: Aplicación en productos lácteos

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La presente Tesis Doctoral, titulada **"POTENCIAL TECNOLÓGICO Y DIGESTIBILIDAD *IN VITRO* DE COPRODUCTOS DEL DÁTIL ILICITANO: APLICACIÓN EN PRODUCTOS LÁCTEOS"** se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

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El Dr. D. *José Ángel Pérez Álvarez*, director, y la Dra. Dña. *Juana Fernández López*, codirectora de la tesis doctoral titulada **"Potencial tecnológico y Digestibilidad *in vitro* de coproductos del dátil ilicitano: Aplicación en productos lácteos"**

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Coordinador del Programa Doctorado ReTos-AAA

Esta Tesis Doctoral se ha desarrollado en el Instituto de Investigación e Innovación Agroalimentaria y Agroambiental de la Universidad Miguel Hernández (CIAGRO-UMH), dentro del Grupo de Investigación de Innovaciones en Productos Alimentarios (IPOA).

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Lista de abreviaturas

ABTS	2,2'-azino-bis(3-etilbenzotiazolina-6-sulfónico)
AGE	Equivalentes de ácido gálico
AGME	Ésteres metílicos de ácidos grasos
AGMI	Ácidos Grasos Monoinsaturados
AGPI	Ácidos Grasos Poliinsaturados
AGS	Ácidos Grasos Saturados
CRA	Capacidad de Retención de Agua
CRO	Capacidad de Retención de Aceite
DCU	Dose Culture Units (Unidades de Dosis de Cultivo)
DPPH	2,2-difenil-1-picrilhidracilo
DP3	Kéfir de cabra con adición de pasta de dátil al 3%
DP4	Queso fresco de cabra con adición de pasta de dátil al 4%
DP6	Kéfir de cabra con adición de pasta de dátil al 6%
DP8	Queso fresco de cabra con adición de pasta de dátil al 8%
ECA	Enzima Convertidora de Angiotensina I
FAO	Food and Agriculture Organization (Organización de las Naciones Unidas para la Alimentación y la Agricultura)
FDT	Fibra Dietética Total
FIC	Método antioxidante por quelación de iones ferrosos
FOS	Fructooligosacáridos
FRAP	Ferric Reducing Antioxidant Power (Poder antioxidante reductor férrico)
HPLC	High-Performance Liquid Chromatography (Cromatografía líquida de alta resolución)
IB	Índice de Blancura
ICP-MS	Inductively Coupled Plasma Mass Spectrometry (Espectrometría de Masas con Plasma Acoplado Inductivamente

Lista de abreviaturas

KHA (1, 2, 3)	Agua de dátiles de maduración khalal
KHH	Harina de dátiles de maduración khalal
KHP	Pasta de dátiles de maduración khalal
MRS	Medio de cultivo de Man, Rogosa y Sharpe
M17	Agar para el recuento de estreptococos lácticos
PAI	Producto Alimentario Intermedio
PBS	Phosphate Buffered Saline (Solución Salina Tamponada con Fosfato)
$p < 0,05$	Nivel de significancia estadística (probabilidad menor al 5%)
RTA (1, 2, 3)	Agua de dátiles de maduración routab
RTH	Harina de dátiles de maduración routab
RTP	Pasta de dátiles de maduración routab
SWC	Swelling Capacity (Capacidad de Hinchamiento)
TMA (1 ,2 3)	Agua de dátiles de maduración tamar
TMH	Harina de dátiles de maduración tamar
TMP	Pasta de dátiles de maduración tamar
UFC	Unidades Formadoras de Colonias
WH25	Kéfir con sustitución de leche de cabra por suero lácteo de queso fresco al 25%
WH50	Kéfir con sustitución de leche de cabra por suero lácteo de queso fresco al 50%
Y3HD	Yogur de cabra con adición de harina de dátil al 3%
Y3PD	Yogur de cabra con adición de pasta de dátil al 3%
Y6HD	Yogur de cabra con adición de harina de dátil al 6%
Y6PD	Yogur de cabra con adición de pasta de dátil al 6%

CAPÍTULO 1. INTRODUCCIÓN



Prólogo

Esta Tesis Doctoral se presenta bajo la modalidad de ***Tesis por compendio de publicaciones*** (8): 1 Capítulo de Libro, 6 artículos de investigación publicados en revistas incluidas en el primer cuartil (Q1) del Journal Citation Report y 1 artículo enviado para su publicación (en revisión).

Publicación 1

Quality characteristics of fresh date palm fruits of "Medjoul" and "Confitera" cv. from the southeast of Spain (Elche palm grove)

Autores: Clara Muñoz-Bas, Nuria Muñoz-Tébar, Laura Candela-Salvador, José Ángel Pérez-Álvarez, José Manuel Lorenzo, Manuel Viuda-Martos, Juana Fernández-López

Revista	Foods
DOI	10.3390/foods12142659
Editorial	MDPI
Categoría de JCR	Food Science and Technology
Cuartil	Q1
Rango	38/173
Factor de impacto (2023)	4,7
Año de publicación	2023

Publicación 2

Development of value-added products suitable for food applications from fresh date fruit (Confitera cv.) and its co-products

Autores: Clara Muñoz-Bas, Nuria Muñoz-Tébar, Laura Candela-Salvador, Estrella Sayas-Barberá, Manuel Viuda-Martos, José Ángel Pérez-Álvarez, Juana Fernández-López

Revista	Food and Bioprocess Technology
DOI	10.1007/s11947-023-03189-9
Editorial	Springer
Categoría de JCR	Food Science and Technology
Cuartil	Q1
Rango	28/173
Factor de impacto (2023)	5,3
Año de publicación	2023

Publicación 3

In vitro evaluation of biological properties of high-added value ingredients (date juice and date powder) obtained from date co-products

Autores: Clara Muñoz-Bas, Rita Vedor, Daniela Machado, Joana Cristina Barbosa, Ana Maria Gomes, José Ángel Pérez-Álvarez, Juana Fernández-López

Revista	Applied Food Research
DOI	10.1016/j.afres.2024.100685
Editorial	Elsevier
Categoría de JCR	Food and Science Technology
Cuartil	Q1
Rango	25/181
Factor de impacto (2024)	6,2
Año de publicación	2024

Publicación 4

Bioactive compounds of fruits, vegetables and their coproducts in the development of dairy functional products

Autores: Clara Muñoz-Bas, Estrella Sayas-Barberá, José Ángel Pérez-Álvarez, Juana Fernández-López, Manuel Viuda-Martos

Libro	Improving health and nutrition through bioactive compounds: Benefits and Applications
ISBN	978-0-443-21873-6 (print) / 978-0-443-21872-9 (online)
Editora	Maira Rubí Segura Campos
Editorial	Elsevier
Ámbito de la publicación	Campo científico
Año de publicación	2024

Publicación 5

Fortification of goat milk yogurts with date palm (*Phoenix dactylifera* L.) coproducts: Impact on their quality during cold storage

Autores: Nuria Muñoz-Tébar, **Clara Muñoz-Bas**, Manuel Viuda-Martos, Estrella Sayas-Barberá, José Ángel Pérez-Álvarez, Juana Fernández-López

Revista	Food Chemistry
DOI	10.1016/j.foodchem.2024.139800
Editorial	Elsevier
Categoría de JCR	Food Science and Technology
Cuartil	Q1
Rango	7/181
Factor de impacto (2024)	9,8
Año de publicación	2024

Publicación 6

Application of date-coproducts for the fortification of fresh goat cheese: Effect on their nutritional, technological, physicochemical, microstructural, microbiological and sensory properties

Autores: Clara Muñoz-Bas, Nuria Muñoz-Tébar, Manuel Viuda-Martos, Estrella Sayas-Barberá, José Ángel Pérez-Álvarez, Juana Fernández-López

Revista	Applied Food Research
DOI	10.1016/j.afres.2024.100619
Editorial	Elsevier
Categoría de JCR	Food and Science Technology
Cuartil	Q1
Rango	25/181
Factor de impacto (2024)	6,2
Año de publicación	2024

Publicación 7

Quality properties of innovative goat milk kefir enriched with date paste (*Phoenix dactylifera* L.) and whey derived from goat cheese production

Autores: Clara Muñoz-Bas, Nuria Muñoz-Tébar, Manuel Viuda-Martos, Raquel Lucas-González, José Ángel Pérez-Álvarez, Juana Fernández-López

Revista	Foods
DOI	10.3390/foods14101655
Editorial	MDPI
Categoría de JCR	Food and Science Technology
Cuartil	Q1
Rango	42/181
Factor de impacto (2023)	5,1
Año de publicación	2025

Publicación 8

Date palm (*Phoenix dactylifera*) and enriched fresh goat cheese: polyphenol profile and stability after INFOGEST 2.0 in vitro digestion

Autores: Raquel Lucas-González, **Clara Muñoz-Bas**, Nuria Muñoz-Tebar, José Ángel Pérez-Álvarez, Manuel Viuda-Martos, Juana Fernández-López

Revista	LWT
DOI	10.1016/j.lwt.2026.119019
Editorial	Elsevier
Categoría de JCR	Food and Science Technology
Cuartil	Q1
Rango	22/181
Factor de impacto (2024)	6,6
Año de publicación	2025

Estructura de la Tesis

La presente Tesis Doctoral se ha realizado siguiendo la estructura de la Normativa de estudios de doctorado de la Universidad Miguel Hernández de Elche para la presentación de "Tesis por compendio de publicaciones". Además, con esta Tesis se pretende obtener la mención de "Doctor Internacional" (art. 15, RD 99/2011 modificado por RD 576/2023).

La Tesis está dividida en 7 capítulos y previamente a ellos, se presenta un resumen/*abstract* de la misma, tanto en español como en inglés. Los 7 capítulos son los siguientes:

Capítulo 1. Introducción

Este capítulo consta de cuatro apartados principales donde se recoge información actualizada sobre la historia del dátil, su producción, su valor nutricional y sus propiedades saludables. Se profundiza en la valorización de los coproductos y el desarrollo de alimentos funcionales, en este caso, productos lácteos. Para finalizar el capítulo, se exponen las principales características de la digestión *in vitro*, los métodos aplicados, y la función de la matriz alimentaria en la bioaccesibilidad de nutrientes y compuestos bioactivos.

Capítulo 2. Objetivos

En este capítulo se presentan tanto el objetivo general como los específicos de la presente Tesis Doctoral.

Capítulo 3. Materiales y Métodos

En este capítulo se especifican los materiales, procesos, protocolos, análisis y métodos aplicados en todos los trabajos que integran la presente Tesis Doctoral. Está dividido en 6 apartados: en los cuatro primeros apartados se expone

todo lo relacionado con la caracterización de los coproductos de dátiles y su valorización mediante la obtención de los productos de alto valor añadido (productos alimentarios intermedios); el quinto capítulo trata sobre el desarrollo de diferentes productos lácteos enriquecidos con dichos productos alimentarios intermedios; y el sexto y último sobre la aplicación de la digestión *in vitro* para determinar la bioaccesibilidad de nutrientes y compuestos bioactivos y el efecto de la matriz alimentaria.

Capítulo 4. Resultados y Discusión

En este capítulo se muestran los resultados más relevantes seleccionados de las publicaciones de investigación que componen la presente Tesis Doctoral. Se divide en 5 apartados, tres de ellos relativos a la caracterización de los coproductos y su valorización mediante la obtención de los productos alimentarios intermedios, otro a su aplicación en el desarrollo de diferentes productos lácteos, y el último a la funcionalidad de las matrices alimentarias desarrolladas.

Capítulo 5. Conclusiones

En este capítulo se recopilan las conclusiones obtenidas de todos los trabajos que forman esta Tesis Doctoral, las cuales se presentan tanto en español como en inglés.

Capítulo 6. Referencias

En este capítulo se recoge toda la bibliografía utilizada para la elaboración de esta Tesis Doctoral.

Capítulo 7. Publicaciones

En este capítulo se incorporan todas las publicaciones científicas que forman parte de esta Tesis, presentadas en el idioma original de la publicación.

La *primera publicación* trata sobre las características morfológicas, propiedades fisicoquímicas, tecnofuncionales y antioxidantes, composición proximal, perfil de azúcares y ácidos orgánicos, y composición de minerales y polifenoles, de los dátiles de los dos cultivares comerciales más abundantes en el Palmeral de Elche (Medjoul y Confitera). A partir de los resultados de la misma se decide seguir trabajando solo con los dátiles del cultivar Confitera.

1. **Muñoz-Bas, C.**, Muñoz-Tébar, N., Candela-Salvador, L., Pérez-Álvarez, JA., Lorenzo, JM., Viuda-Martos, M., Fernández-López, J. *Quality Characteristics of Fresh Date Palm Fruits of "Medjoul"and "Confitera" cv. from the Southeast of Spain (Elche Palm Grove)*. Foods, MDPI, 2023, 12, 2659. https://doi.org/10.3390/foods12142659

Las dos publicaciones siguientes (*publicaciones 3 y 4*) tratan sobre el procesamiento de los coproductos generados de la industrialización de los dátiles Confitera para la obtención de productos de alto valor añadido (Productos Alimentarios Intermedios.-PAI), y su caracterización (composición proximal, perfil de azúcares y ácidos orgánicos, perfil de minerales, propiedades fisicoquímicas y tecnofuncionales, análisis microbiológico y propiedades biológicas, que incluyen, actividad prebiótica, antidiabética, antihipertensiva y antioxidante).

2. **Muñoz-Bas, C.**, Muñoz-Tébar, N., Candela-Salvador, L., Sayas-Barberá, E., Viuda-Martos, M., Pérez-Álvarez, JA., Fernández-López, J. *Development of Value-Added Products Suitable for Food Applications from Fresh Date Fruit (Confitera cv.) and its co-products*. Food and Bioprocess Technology, 2024,17, 1265-1277. https://doi.org/10.1007/s11947-023-03189-9
3. **Muñoz-Bas, C.**, Vedor, R., Machado, D., Barbosa, JC., Gomes, AM., Pérez-Álvarez, JA., Fernández-López, J. *In vitro evaluation of biological properties of high-added value ingredients (date juice and date powder) obtained from date co-products*. Applied Food Research, 2025, 5, 1, 100685. https://doi.org/10.1016/j.afres.2024.100685

La *cuarta publicación* es una revisión bibliográfica que compone uno de los capítulos del libro titulado "Improving health and nutrition through bioactive compounds: Benefits and applications" que se centra en la revisión del estado del arte sobre el desarrollo de productos lácteos con la adición de compuestos bioactivos procedentes de frutas, verduras y sus coproductos.

4. **Muñoz-Bas, C.**, Sayas-Barberá, E., Pérez-Álvarez, JA., Fernández-López, J., Viuda-Martos, M. *Bioactive compounds of fruits, vegetables and their co-products in the development of dairy functional products*. Elsevier, ISBN 9780443218729 (online) y 9780443218736 (print).

La *quinta publicación* se dedica al desarrollo, caracterización y vida útil de un producto lácteo, yogur en este caso, al cual se le adicionó un Producto Alimentario. Intermedio (PAI) obtenido del procesamiento de los coproductos del dátil Confitera.

5. Muñoz-Tébar, N., **Muñoz-Bas, C.**, Viuda-Martos, M., Sayas-Barberá, E., Pérez-Álvarez, JA., Fernández-López, J. *Fortification of goat milk yogurts with date palm (Phoenix dactylifera L.) co-products: Impact on their quality during cold storage*. Food Chemistry, 2024, 454, 139800. https://doi.org/10.1016/j.foodchem.2024.139800

La *sexta publicación* se centra en la elaboración de un queso fresco de cabra con adición de pasta de dátil Confitera, al cual se le evaluaron las propiedades nutricionales, tecnológicas, fisicoquímicas, microbiológicas, microestructurales, composición de minerales, azúcares y ácidos orgánicos y se sometió a análisis sensorial.

6. **Muñoz-Bas, C.**, Muñoz-Tébar, N., Viuda-Martos, M., Sayas-Barberá, E., Pérez-Álvarez, JA., Fernández-López, J. *Application of date co-products for the fortification of fresh goat cheese: Effect on their nutritional, technological, physicochemical, microstructural, microbiological and*

sensory properties. Applied Food Research, 2024, 4, 2, 100619. https://doi.org/10.1016/j.afres.2024.100619

La *séptima publicación* estudia la elaboración de un kéfir de cabra con adición de pasta de dátil y suero lácteo procedente del queso fresco con adición del PAI "pasta de dátil", al que se le analizaron las propiedades nutricionales, tecnológicas, fisicoquímicas, microbiológicas, perfil de minerales, azúcares y ácidos orgánicos, y se sometió a análisis sensorial.

7. **Muñoz-Bas, C.**, Muñoz-Tébar, N., Viuda-Martos, M., Lucas-González, R., Pérez-Álvarez, JA., Fernández-López, J. Quality properties of innovative goat milk kefir enriched with date paste (Phoenix dactylifera L.) and whey derived from goat cheese production. Foods, MDPI, 2025, 14, (10). https://doi.org/10.3390/foods14101655

La *octava publicación* es un artículo científico sobre la digestibilidad *in vitro* de uno de los productos alimentarios intermedios obtenidos de la valorización de los coproductos del dátil, en este caso, pasta de dátil, y del queso fresco fortificado con él.

8. Lucas-González, R., **Muñoz-Bas, C.**, Muñoz-Tebar, N., Pérez-Álvarez, JA., Viuda-Martos, M., Fernández-López, J. Date palm (*Phoenix dactylifera*) and enriched fresh goat cheese: polyphenol Profile and stability after INFOGEST 2.0 in vitro digestion method. LWT, Elsevier, 2026. https://doi.org/10.1016/j.lwt.2026.119019

Resumen

La producción sostenible de alimentos, junto con el desarrollo de productos que contribuyan activamente a la promoción de la salud, constituye en la actualidad un eje prioritario tanto en el ámbito científico como en el social. En este contexto, los coproductos generados por la industria agroalimentaria —como pieles, pulpas, semillas u hojas— representan una valiosa fuente de nutrientes y compuestos bioactivos. Su adecuada recuperación y procesamiento permite su reintroducción en la cadena alimentaria, favoreciendo la elaboración de nuevos productos con mayor valor añadido y sostenibilidad.

El presente trabajo se centra en la valorización de coproductos del dátil confitera (*Phoenix dactylifera* L.) mediante su transformación en productos alimentarios intermedios, como pasta, agua y harina de dátil, con el objetivo de promover estrategias de economía circular y sostenibilidad en la industria agroalimentaria. El objetivo general de esta Tesis Doctoral es aportar datos científicos que permitan a las industrias comercializadoras de dátiles frescos aprovechar al máximo la cosecha de este fruto, no solo mediante su comercialización directa, sino también a través de la valorización de los coproductos generados. De esta forma, se busca obtener ingredientes de alta calidad nutritiva y sanitaria, suficientemente estables para ser utilizados por la industria alimentaria —en particular, la industria láctea— en el desarrollo de productos más saludables y sostenibles.

Para ello, los productos intermedios obtenidos fueron incorporados en diferentes matrices lácteas elaboradas con leche de cabra (yogur, queso fresco y kéfir), que fueron caracterizadas en cuanto a su composición fisicoquímica, valor nutricional, contenido mineral, ácidos orgánicos, azúcares, propiedades texturales y atributos sensoriales. Asimismo, se evaluó el perfil y la estabilidad de los (poli)fenoles libres y unidos mediante digestión gastrointestinal *in vitro*, tanto en la pasta de dátil como en el queso fresco enriquecido con esta.

Los resultados mostraron que la incorporación de coproductos de dátil mejoró el perfil nutricional y funcional de los productos lácteos sin comprometer su aceptación sensorial. Además, la digestión *in vitro* reveló una alta estabilidad y

bioaccesibilidad de diversos compuestos fenólicos, especialmente flavonoles y ácidos hidroxicinámicos, confirmando el potencial del dátil como ingrediente funcional.

En conjunto, este estudio demuestra que la valorización de coproductos del dátil confitera constituye una estrategia viable para el desarrollo de alimentos lácteos funcionales y sostenibles, contribuyendo a la reducción del desperdicio alimentario y al aprovechamiento integral de los recursos agroalimentarios.

Abstract

The sustainable production of food, together with the development of products that actively promote health, currently represents a key priority in both scientific and social contexts. In this regard, coproducts generated by the agri-food industry—such as peels, pulps, seeds, and leaves—constitute valuable sources of nutrients and bioactive compounds. Their proper recovery and processing enable their reintegration into the food chain, supporting the development of new products with higher added value and improved sustainability.

The present work focuses on the valorization of co-products derived from confectionery dates (*Phoenix dactylifera* L.) through their transformation into intermediate food products, such as date paste, date water, and date flour, with the aim of promoting circular economy and sustainability strategies within the agri-food sector. The main objective of this Doctoral Thesis is to provide scientific evidence that enables the date commercialization industry to maximize the use of the harvested fruit, not only through the sale of fresh dates but also by valorizing the generated coproducts. In this way, it seeks to obtain nutritionally and hygienically high-quality ingredients, sufficiently stable to be used by the food industry—particularly the dairy sector—in the development of healthier and more sustainable products.

For this purpose, the intermediate products obtained were incorporated into different goat milk–based dairy matrices (yogurt, fresh cheese, and kefir), which were characterized in terms of their physicochemical composition, nutritional

value, mineral content, organic acids, sugars, textural properties, and sensory attributes. Additionally, the profile and stability of free and bound (poly)phenols were evaluated through in vitro gastrointestinal digestion, both in the date paste and in the fresh cheese enriched with it.

The results showed that the incorporation of date coproducts improved the nutritional and functional profile of the dairy products without compromising their sensory acceptance. Furthermore, *in vitro* digestion revealed high stability and bioaccessibility of various phenolic compounds—particularly flavonols and hydroxycinnamic acids—confirming the potential of date-derived ingredients as functional components.

Overall, this study demonstrates that the valorization of date coproducts is a viable strategy for developing functional and sustainable dairy foods, contributing to the reduction of food waste and the integral use of agri-food resources.

1. INTRODUCCIÓN

1.1. EL DÁTIL *(Phoenix dactylifera L.)*

1.1.1. Historia y producción

El dátil, fruto de la palmera datilera (*Phoenix dactylifera L.*), es uno de los cultivos frutales más antiguos debido a que proviene de ciertas regiones conocidas como "mundo antiguo" desde hace al menos 5000 años A.C. Es cierto que se desconoce su procedencia exacta, pero hay indicios de que su origen sea en la antigua zona de Mesopotamia, actualmente conocida como Irak (Chao y Krueger, 2007). Además, destaca su gran importancia cultural, religiosa, social, medioambiental, de subsistencia y económica en muchos países. Principalmente, el dátil es más abundante en zonas con un clima cálido y seco donde los veranos son largos y calurosos, de escasas precipitaciones y baja humedad, es decir, en regiones áridas o semiáridas. Tradicionalmente, estas regiones han pertenecido a Oriente Medio y el Norte de África, pero con el paso del tiempo, se ha ido introduciendo en otros continentes y países como Estados Unidos (California y Arizona), Perú y Australia (Echegaray et al., 2020, 2021; Fernández-López et al., 2022).

En la actualidad, los mayores productores de palmeras datileras siguen siendo las zonas del norte de África y Oriente Medio, en concreto Egipto, Arabia Saudí, Argelia, Irán e Irak (figura 1). Egipto es el primer productor mundial con más 1,8 millones de toneladas al año. Siguiendo a los países nombrados anteriormente se encuentran Marruecos, Túnez, China, Libia y Omán con una producción entorno a las 400 mil toneladas. En el caso concreto de España, tiene una producción de palmeras datileras alrededor de las 14600 toneladas al año.

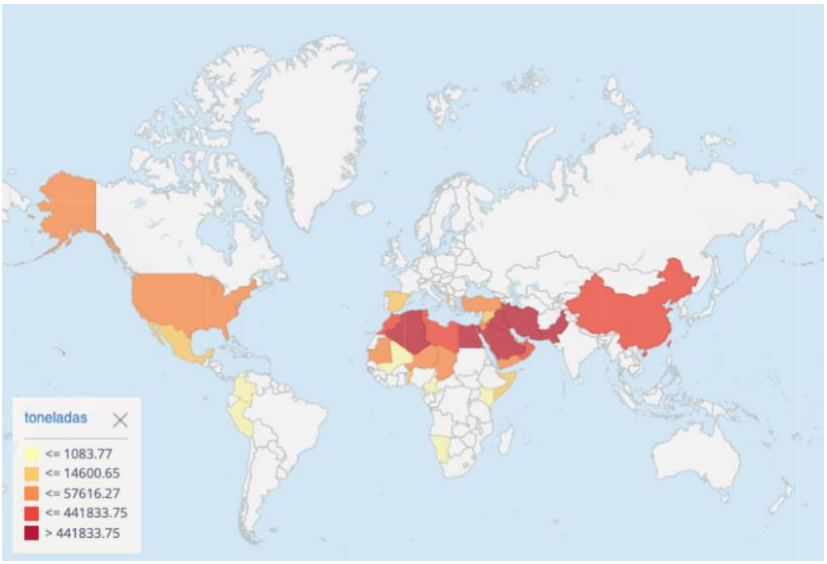


Figura 1. Producción mundial de dátiles (toneladas) procedente de palmera datilera (*Phoenix Dactylifera*) en 2023. Fuente: FAOSTAT (2025).

Botánicamente hablando, la palmera datilera (*Phoenix dactylifera L.*) pertenece a la familia Arecaceae y al género *Phoenix*, siendo la palmera datilera un árbol dioico. El dátil es una drupa, es decir, un fruto carnoso y con hueso en su interior. La polinización es muy importante tanto en la producción de los frutos, como en la mejora de la calidad y cantidad de los mismos (Shahsavar y Shahhosseini, 2021). En cuanto al proceso de polinización, de acuerdo con Ourani-Pourdashti et al. (2024), se pueden encontrar dos categorías:

1. **Polinización externa natural**, la cual se lleva a cabo mediante agentes bióticos y abióticos.
2. **Polinización externa artificial** (macheado), la cual se produce a través de métodos diseñados por el hombre, ya sean manuales o mecánicos.

A pesar de existir un método natural, si hablamos de eficiencia y calidad en cuanto a la polinización y la producción comercial de dátiles, es significativamente más eficiente la polinización externa artificial (Ourani-Pourdashti et al., 2024). Además, este tipo de polinización se lleva investigando desde mediados del siglo pasado con el objetivo de conseguir el método más eficiente, ya sea utilizando dispositivos mecánicos, motorizados o eléctricos (Shahsavar y Shahhosseini, 2021).

Tras la polinización, los dátiles sufren una serie de cambios en cuanto al color, textura, olor y sabor, atravesando así cinco fases de desarrollo (figura 2) (Mohammed et al., 2021; El-Beltagi et al., 2023):

1. **Hababuk**: corresponde con la fase inicial, las primeras cinco semanas de desarrollo, donde el fruto es diminuto e inmaduro.
2. **Kimri**: equivale a las semanas de la seis a la dieciséis. Aquí el fruto es verde, con gran cantidad de agua y pocos azúcares simples.
3. **Khalal**: corresponde al período comprendido entre las semanas diecisiete a la veinte. En esta fase el fruto comienza a cambiar de color, pasando del verde a tonalidades más amarillas.
4. **Rutab**: de la semana veintiuno a la veinticuatro. En esta fase, el fruto comienza a madurar, con un contenido en agua en torno al 30-45%.
5. **Tamar**: comprende las semanas de la veinticinco a la veintisiete, es la fase final. Aquí, el fruto es de color marrón oscuro, menos agua (en torno al 10-25%) y puede ser blando o duro.

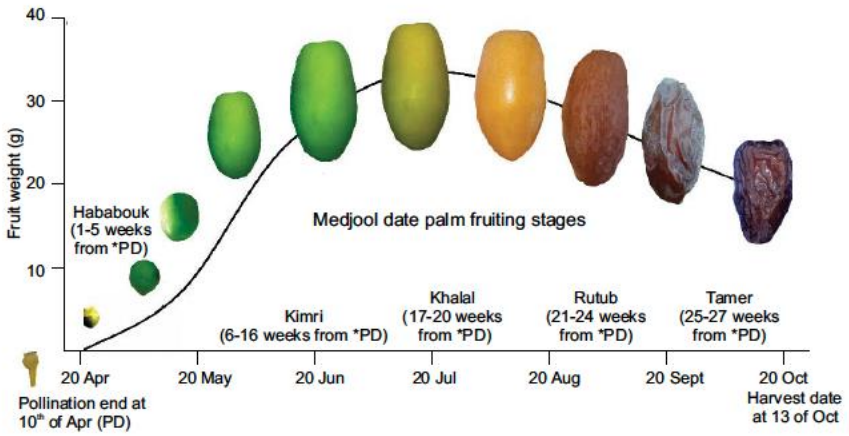


Figura 2. Diferentes etapas de maduración del dátil Medjoul desde la polinización hasta la cosecha. (Fuente: Al-hajjaj y Ayad, 2018).

1.1.2. El dátil en España

El dátil fue introducido en España hace más de 2500 años por los fenicios griegos y romanos, mucho antes de la llegada de los árabes. A pesar de esto, es cierto que fue debido a la expansión del Islam y la llegada de los árabes cuando se instauró dicho cultivo y las prácticas que rigen en la actualidad. Por ello, los

españoles fueron los primeros en introducir la palmera datilera fuera de las zonas de la Península Arábiga, el norte de África, y Oriente Medio (Chao y Krueger, 2007).

Tradicionalmente, el dátil se utilizaba como producto de intercambio y comercio interno. En la actualidad es la variedad Medjoul la que más se comercializa debido a su alto valor comercial, al ser dátiles secos y semiblandos y su fácil conservación con respecto a los dátiles frescos. Hace décadas, la producción anual de dátiles en España rondaba las 14 mil toneladas (figura 3). Con el paso del tiempo, esta producción comenzó a disminuir, alrededor de los años 70 y 80, llegando a ser a una producción de apenas unas 2 mil toneladas en el año 2020. Esta disminución se debió a que alrededor de esas décadas, muchos alimentos fueron despreciados injustamente por diversos factores, como el contenido en grasa (pescados grasos, aceite de oliva...), en almidón (pan, patatas...) y azúcares (varios tipos de frutas, en especial el dátil). Gracias a la ciencia y a diversos estudios rigurosos, estas afirmaciones erróneas pudieron impugnarse de forma contundente (Pérez-Álvarez et al., 2023).

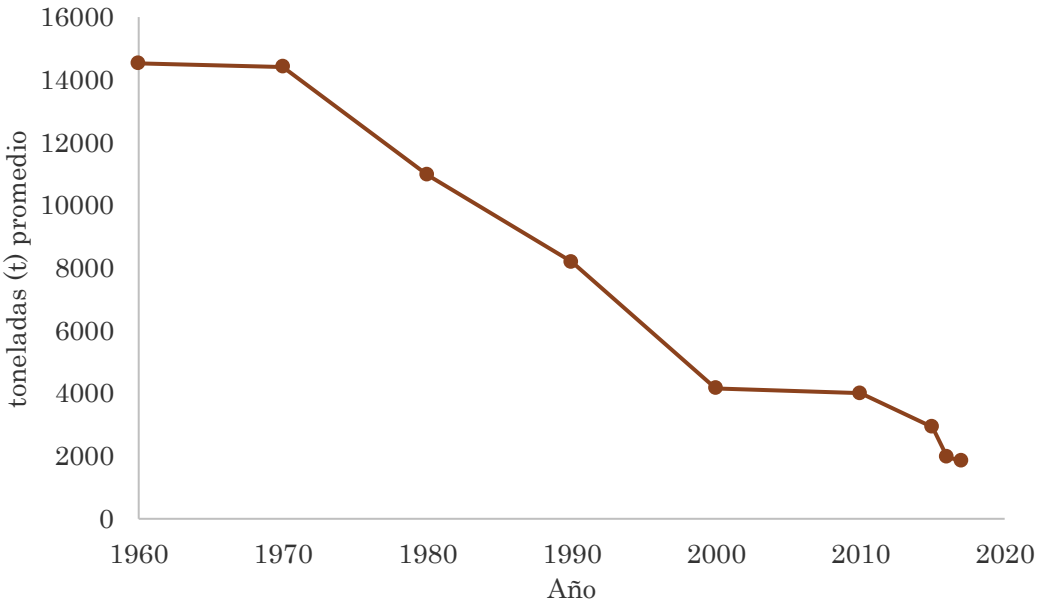


Figura 3. Producción de dátiles (toneladas) a lo largo del tiempo en España. Elaboración propia. Fuente: MAPAMA (2025).

A pesar de que la producción de dátiles ha ido decreciendo a lo largo de los años, en el sureste de España, particularmente en la ciudad de Elche (Alicante,

España) es donde se encuentra el palmeral más grande de toda Europa. Este palmeral destaca por tener aproximadamente 500 hectáreas y alrededor de 200.000 ejemplares de palmeras censadas, oficialmente, siendo declarado por la UNESCO Patrimonio de la Humanidad en el año 2000. Cuenta con una gran diversidad de cultivares siendo, comercialmente, el "Medjoul" y el "Confitera" los dos más destacados, De la producción total (comestible) de dátil ilicitano, solo se industrializa el 0,02% (Martín-Sanchéz et al., 2014).



Figura 4. Dátiles secos Medjoul procedentes del palmeral de Elche.
Fuente: Todopalmera.com (https://sl1nk.com/pwcjy)



Figura 5. Dátiles frescos Confitera procedentes del palmeral de Elche.
Fuente: Todopalmera.com (https://sl1nk.com/Z6LMM)

Principalmente, existe una gran diferencia entre ambas variedades, puesto que los dátiles Medjoul se comercializan secos (figura 4) y los Confitera frescos (figura 5). Además, comercialmente, el cultivar Confitera es el único autóctono, del palmeral ilicitano, lo que contribuye a proteger la biodiversidad nacional y reforzar la economía circular, reduciendo también la huella de carbono, ya que permite utilizar no solo el fruto principal, sino también sus coproductos en recursos útiles, reduciendo pérdidas, generando empleo local y aportando sostenibilidad a la cadena agroalimentaria. Además, cabe destacar que el 15 de mayo de 2023 la Conselleria de Agricultura, Desarrollo Rural, Emergencia Climática y Transición Ecológica aprobó la reglamentación de calidad del dátil de Elche para su distinción con la marca de calidad CV, lo que certifica que el dátil es un producto agroalimentario producido, transformado o elaborado en la Comunidad Valenciana cumpliendo unos estándares de calidad superior que refuerza la confianza del consumidor y el valor del producto en el mercado (Generalitat Valenciana, 2023).

1.1.3. Composición química

Teniendo en cuenta que el dátil ilicitano se comercializa fresco, lo que lo hace diferir bastante, tanto en su composición como en su vida útil con respecto al dátil "seco". Así, los datos de composición del dátil fresco ilicitano no aparecen en las tablas nutricionales "oficiales" de Base de Datos Española de Composición de Alimentos (BEDCA). De forma genérica, el dátil seco, es una fruta que destaca por ser rica, principalmente, en azúcares, fibra, minerales y compuestos bioactivos. En la tabla 1, se puede comprobar los valores de la composición nutricional del dátil seco, en la cual, como componente mayoritario, se encuentran los azúcares, seguidos del agua y la fibra dietética.

Tabla 1. Composición química del dátil seco (Fuente: BEDCA).

Composición nutricional (por 100 g)	
Parte comestible (%)	90
Energía (Kcal)	288
Agua (g)	16,5
Proteínas (g)	2,5
Grasas (g)	0,5
Carbohidratos (g)	67
Fibra (g)	7,1
Vitamina A* (µg)	5
Vitamina E** (mg)	2,2
Folato, total (µg)	28
Niacina*** (mg)	1,7
Riboflavina (mg)	0,1
Tiamina (mg)	0,06
Vitamina B6 (mg)	0,2
Vitamina C (mg)	2
Calcio (mg)	62
Hierro (mg)	3
Potasio (mg)	677
Magnesio (mg)	58
Sodio (mg)	3
Fósforo (mg)	74
Selenio (µg)	3
Zinc (mg)	0,3

Fuente: Base de datos española de composición de alimentos (BEDCA, 2025).

Además, de acuerdo con el Reglamento (CE) Nº 1924/2006 del Parlamento Europeo y del Consejo del 20 de diciembre de 2006, el dátil, al contener más de 6 g de fibra por 100 g de producto, se puede declarar como un alimento con alto

contenido en fibra dietética (Parlamento Europeo, 2006). Los dátiles también destacan por su contenido en minerales (K, Ca, Mg, P), vitaminas, compuestos fenólicos, antocianinas, carotenoides y esteroles (El-Far et al., 2019), otorgándole a los dátiles propiedades saludables y funcionales (El-Arem et al., 2017; Echegaray et al., 2020, 2021; Fernández-López et al., 2022; Ourani-Pourdashti et al. 2024). No obstante, hay que tener en cuenta que, al igual que el resto de las frutas, su composición está estrechamente relacionada con la variedad, la fase de maduración, la recolección y post-recolección.

1.1.4. Efectos saludables

Actualmente, el dátil sigue siendo un gran desconocido nutricionalmente hablando, para buena parte de la población mundial. Este fruto tiene un gran perfil nutricional (como se ha visto en el apartado anterior), destacando el contenido en azúcares, fibra dietética, vitaminas esenciales y minerales, por ejemplo, en el cultivar Confitera, su contenido en potasio, es casi tres veces superior (677 mg/100 g) al contenido presente en el plátano (350 mg/100 g). También cuenta con numerosos compuestos bioactivos (polifenoles, antocianinas, esteroles y carotenoides) (Echegaray et al., 2021).

Los compuestos fenólicos presentan una estructura química capaz de neutralizar radicales libres. Además, estos compuestos fenólicos, debido a sus características, tienen beneficios para la salud como propiedades antibacterianas, antihiperlipidémicas, anticancerígenas, antioxidantes, cardioprotectoras, neuroprotectoras y antidiabéticas (Khoddami et al., 2013; Lin et al., 2016; Al Mamari 2022).

- *Actividad antioxidante:* cabe destacar que hay una estrecha correlación entre el contenido de fenoles y la actividad antioxidante; a mayor contenido en compuestos fenólicos, presentan una mayor actividad antioxidante (Muflihah et al., 2021). Además, de acuerdo con varios autores, el dátil es una gran fuente de compuestos fenólicos, los cuales se encuentran

principalmente en la pulpa y en la semilla (Khatib et al 2022; Alsuhaymi et al 2023).

- *Actividad antidiabética:* la diabetes es una enfermedad metabólica crónica, la cual está relacionada con niveles muy elevados de glucosa que pueden provocar daños en diversos órganos, para ello se buscan inhibidores de la enzima α-glicosidasa, la encargada de transformar los oligo- y di- sacáridos en monosacáridos, facilitando la absorción de hidratos de carbono y elevando los niveles de insulina. Por otro lado, se ha demostrado que existe una estrecha relación entre el contenido en polifenoles y las propiedades antidiabéticas, lo que quiere decir que, a mayor presencia en compuestos polifenólicos, mayor es la actividad antidiabética (Wu et al., 2015). Además, existen numerosos estudios donde se ha observado que los dátiles tienen efectos antidiabéticos relacionados por su contenido en compuestos bioactivos, los cuales inhiben a la enzima α-glicosidasa (Khan et al., 2016; El Abed et al., 2017; Evans et al., 2018; Mia et al., 2020).

- *Actividad prebiótica:* los prebióticos destacan por sus efectos protectores sobre el sistema gastrointestinal relacionados con la regulación de las bacterias intestinales. También pueden reducir los niveles de lípidos en sangre, mejorar la resistencia a la insulina y aumentar la biodisponibilidad de minerales (Gibson et al., 2017; Davani-Davari et al., 2019; Faustino et al., 2023). Además, existen estudios donde se ha observado dicha actividad prebiótica en semillas de dátil, donde se logró estimular el crecimiento de ciertas cepas de *Lactobacillus*, como *L. paracasei* o *L. casei* (Al-Thubiani y Khan, 2017).

- *Actividad antihipertensiva:* la hipertensión es uno de los principales factores de riesgo para el desarrollo de enfermedades cardiovasculares, según la Organización Mundial de la Salud (OMS, 2023), uno de cada tres adultos en el mundo padece hipertensión. Además, su prevalencia se duplica en personas con diabetes en comparación con la población no diabética

(Farida et al., 2023). Una de las estrategias terapéuticas más empleadas para controlar la hipertensión es la inhibición de la enzima angiotensina I (ECA), enzima clave en la regulación de la presión arterial ya que reduce la vasoconstricción (Faustino et al., 2023; OMS 2023). Existen diversos estudios que afirman que esta actividad antihipertensiva se debe principalmente a la presencia en estos frutos de flavonoides, vitaminas, minerales y compuestos bioactivos como ácido láurico, ácido linolénico, ácido palmítico, tocoferoles, β-sitosterol e isosorbida (Obode et al., 2020; Yousefi et al., 2021).

1.1.5. Industrialización del dátil

El dátil se conoce principalmente por su consumo y comercialización del fruto entero, ya sea seco o fresco. Además, es un alimento muy versátil que puede utilizarse, como ingrediente, para la elaboración de prácticamente cualquier alimento. Se han incorporado dátiles (figura 6), como suplementación en la elaboración de productos lácteos (Hamdia y Al-Hamdani, 2016), o en productos de panadería como galletas (Shabnam et al., 2020), cereales (Bchir et al., 2018) y bizcochos veganos (Elkatry et al., 2024), productos cárnicos... El dátil es ideal para la elaboración de este tipo de alimentos debido tanto a su contenido en azúcares como de fibra dietética (Elkatry et al., 2024; Barathikannan et al., 2025).

Por otro lado, la semilla de dátil, que destaca por ser rica en fibra dietética, fenoles y proteínas, se utiliza para la elaboración de aceite de semilla de dátil (Mrabet et al., 2024). El aceite de semilla de dátil es reconocido por ser una gran fuente de ácidos grasos insaturados, principalmente ácido oleico y linoleico (Nehdi et al., 2018). Entre otras múltiples aplicaciones, también se puede utilizar la harina de semilla de dátil para la elaboración de galletas (Elsamahy et al., 2024) e incluso para la elaboración de productos cárnicos (Hosseini et al., 2014).

A pesar de esto, la industrialización del dátil sigue siendo bastante limitada, centrada principalmente, en su comercialización como dátil fresco o seco y en su

aplicación en el desarrollo de algunos alimentos por parte de productores locales y explotaciones pequeñas. Esto, desde el punto de vista agroalimentario, podría considerarse como un potencial infrautilizado. Los productos alimentarios intermedios generados durante la industrialización del dátil (dátiles de destrío, que no cumplen las especificaciones comerciales y de calidad de la Marca de Calidad de la CV Dátil de Elche, para su venta, así como los dátiles con algunos daños superficiales por golpes, parásitos, etc., o incluso en estados de maduración no aptos para su consumo) pueden procesarse adecuadamente para la obtención de productos de alto valor añadido (manteniendo las características nutricionales y funcionales de los dátiles de procedencia), conservados de forma adecuada y estables en el tiempo, que pueden usarse como ingredientes en el desarrollo de alimentos funcionales. Además, supone la valorización de los coproductos del dátil, que de otra forma serían desechados. Algunos de estos productos obtenidos a partir del dátil son:

- *Zumo de dátil:* se produce mediante la molienda húmeda de los dátiles frescos, obteniendo una pasta que se prensa, se pasteuriza a 80ºC y se centrifuga, dando lugar como resultado final un zumo de dátil (Kulkarni et al., 2010), el cual destaca por su contenido en sólidos solubles totales (19,5%) y en azúcares (18,3%).

- *Sirope de dátil:* los siropes pueden definirse como zumos concentrados. El sirope de dátil, debido a sus propiedades, podría utilizarse como sustituto de la sacarosa (Abu, 2002). Además, también destaca por su contenido en minerales, como son potasio, calcio, magnesio, fósforo y zinc (Lachman et al., 2010).

- *Pasta de dátil:* se obtiene a partir de triturar la pulpa del dátil. Esta destaca principalmente por su contenido en humedad (35%), en azúcares totales (53%), en fibra dietética (7%), en minerales y antioxidantes naturales (Sánchez-Zapata et al., 2011; Martín-Sánchez et al., 2014).

- *Concentrados de fibra de dátil:* estos coproductos se obtienen mediante la extracción con agua caliente de la pulpa de dátil con un contenido en fibra dietética entre 37-75% dependiendo de la variedad y estado de maduración (Borchani et al., 2010; Hasnaoui et al., 2012). El cual destaca, además de por su valor nutricional, por sus propiedades tecnológicas y funcionales, debido a una alta capacidad de retención de agua y aceite, permitiendo su uso como ingrediente en alimentos (Hasnaoui et al., 2012).

- *Harina de dátil:* destaca por su fácil manipulación e incorporación en las distintas matrices alimentarias. Esta harina se consigue mediante el secado y la molienda de los frutos, resultando con un contenido en humedad entre 2,1-3,7% (Hasan et al., 2022).

- *Extractos de dátil:* la obtención de estos extractos se consigue a partir de la pulpa de dátil o de su polvo desgrasado, mediante la utilización de diferentes solventes como agua, metanol, etanol, acetona, etc. Estos extractos destacan por su aplicación como antioxidantes o antimicrobianos debido a su presencia de compuestos fenólicos y flavonoides (Kchaou et al., 2013; de la Rosa- Alcaraz et al., 2017; Al-Shwyeh, 2019).

- *Aceite de semilla de dátil:* este coproducto cuenta con un alto interés debido a su perfil lipídico (ácido oleico 40-50%, ácido linoleico 8-19%, ácido láurico, 18-50% y ácido palmítico 10-15%) y su alto contenido en fenoles, tocoferoles y fitoesteroles (Besbes et al., 2004; Mrabet et al., 2019; Harkat et al., 2022). Esto lo convierte en un ingrediente de alto valor nutricional para su aplicación en alimentos.

La disponibilidad de dichos ingredientes constituiría un gran avance para la industria alimentaria, ya que podrían utilizarse para la innovación de alimentos, desarrollando productos más saludables y funcionales, contribuyendo a su vez a una economía más sostenibles e involucrada con el medio ambiente. En la figura 6, se observan algunos alimentos a base de dátil que podemos encontrar en

España, como sirope, pasta, azúcar, licor o alimentos elaborados con dátil como yogures, chocolate, galletas, dátiles con bacon entre otros. Además, también se pueden encontrar alimentos tradicionales árabes que contienen dátil.

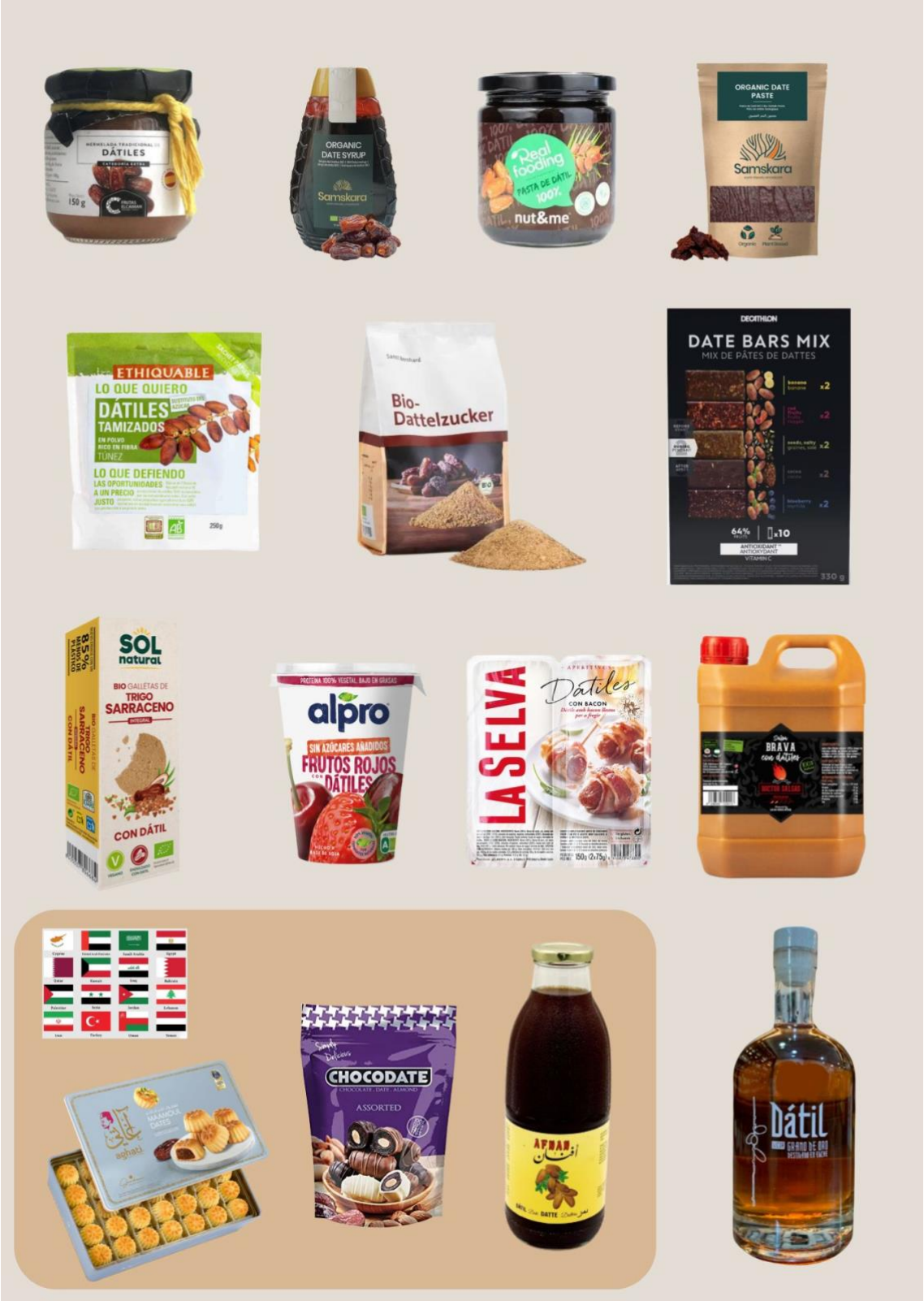


Figura 6. Alimentos elaborados con dátil.

1.2. INDUSTRIA ALIMENTARIA: VALORIZACIÓN DE COPRODUCTOS

En la actualidad, tanto la población mundial como la industria alimentaria esta más comprometida con el medio ambiente y la práctica de una Economía Circular. Existen numerosas definiciones de Economía Circular (Kirchherr et al., 2023), pero se podría resumir como, un modelo económico en el que se busca aprovechar al máximo los recursos, productos y materiales, reduciendo los residuos todo lo posible y fomentar, de esta forma, el reciclaje, la reutilización y la valorización de dichos alimentos (Kirchherr et al., 2017).

Por otro lado, el desperdicio de alimentos desde un punto de vista económico podría definirse como un despilfarro de recursos. Desde un punto de vista social, acentúa los problemas de inseguridad alimentaria debido a los millones de personas que padecen hambre en el mundo. Y desde un punto de vista medioambiental, aporta más gases de efecto invernadero a la atmósfera (Ezeorba et al., 2024).

Solo en Estados Unidos, el desperdicio se encuentra entre el 30% y el 40%, por lo que abordar este desperdicio alimentario es crucial para promover la sostenibilidad, mitigar el impacto medioambiental y fomentar la distribución equitativa (Ezeorba et al., 2024). Mientras, en España, el desperdicio alimentario de 2023 se ha incrementado en un 1,1% con respecto a 2022, siendo un total de 1214,76 millones de kg durante 2023, por lo que 7 de cada 10 hogares desperdicia alimentos. A pesar de que en 2023 el desperdicio alimentario se ha incrementado, es cierto que durante la pandemia del COVID 19 en el año 2020, el desperdicio comenzó a disminuir, consiguiendo en 2022 una disminución de un 13,2% (figura 7).

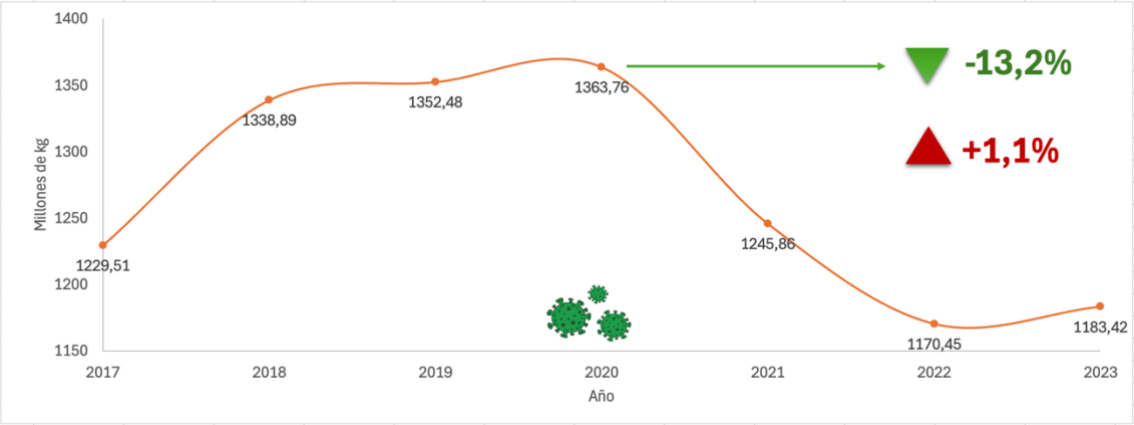


Figura 7. Desperdicio alimentario total (millones de kg) en España a lo largo de los años 2017-2023 (MAPAMA, 2025)

Además, el deficiente aprovechamiento de los alimentos en España en 2023 se incrementó principalmente en la leche, las legumbres y la carne fresca (figura 8).

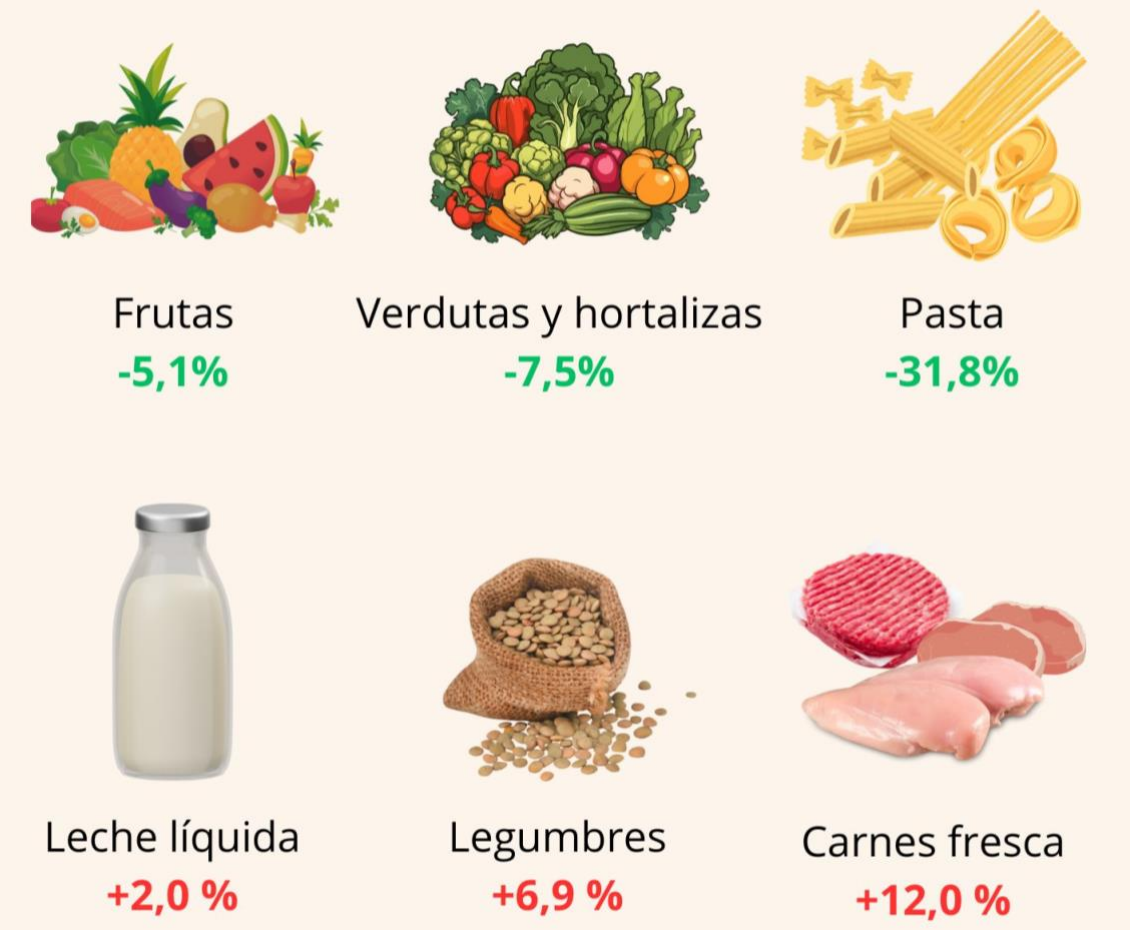


Figura 8. Comparativa (aumento y disminución) del desperdicio alimentario en España en los años 2022-2023 del desperdicio alimentario español según el grupo de alimento (MAPAMA, 2024)

El desperdicio en España es un problema real, tanto dentro como fuera de los hogares, aunque hay que tener en cuenta que el desperdicio fuera del ámbito doméstico es bastante mayor, siendo en 2023 de un 0,8 % con respecto al desperdicio doméstico que alcanzó el 4,4 %. Una de las formas que se utilizan para la concienciación de la población con respecto a este problema, es la creación de infografías (figura 9) que tienen como fin servir de aprendizaje para disminuir el desperdicio y conseguir una economía más sostenible.



Figura 9. Infografía del desperdicio alimentario fuera del hogar realizada por el Ministerio de Agricultura, Pesca y Alimentación (MAPAMA, 2021).

Por todo esto, la valorización de coproductos procedentes de los alimentos no es solo una forma de reducir el desperdicio de éstos, también es una forma de mejorar la sostenibilidad y contribuir al cumplimiento de algunos de los Objetivos de Desarrollo Sostenible (ODS) establecidos por las Naciones Unidas el 25 de septiembre de 2015. Algunos de ellos serían el ODS nº 12, en el cual se impulsa un consumo y producción responsables, o el ODS nº 13, en el que se busca reducir las emisiones de gases de efecto invernadero procedentes de los residuos alimentarios (Hassoun, 2025).

Estos coproductos pueden generarse durante las diferentes fases del procesamiento del alimento, tanto en la fase relacionada con la agricultura como en las fases del procesado del mismo. Además, se ha demostrado que este tipo de productos, que comúnmente se denominan “desperdicios”, una palabra muy desafortunada puesto que destacan por contener una gran variedad de

compuestos nutricionales, funcionales y bioactivos (Ratu et al., 2023). Por ejemplo, destacan por ser fuentes de minerales (K, Ca, Mg, Mn y Fe), fibra dietética, compuestos fenólicos (entre 16,18 mg de EAG/ g y 18,78 mg EAG/ g de fenoles totales) y antioxidantes entre otros (Oladzad et al., 2021).

En referencia al dátil, de acuerdo con la FAO (FAO, 2023), se producen en todo el mundo unos 9 millones de toneladas de dátiles, de los cuales se descarta alrededor del 30% por no considerarse aptos para su comercialización debido a su tamaño, color, infestaciones o daños naturales, entre otros. Por esto, en la presente tesis se ha optado por aplicar a los dátiles una serie de tratamientos y procesos con el objetivo de conseguir coproductos de alto valor nutricional y viables tecnológicamente hablando para su incorporación en alimentos.

1.3. DESARROLLO E INNOVACIÓN DE ALIMENTOS: PRODUCTOS LÁCTEOS

1.3.1. Desarrollo e innovación alimentaria. Alimentos funcionales

Actualmente, la industria alimentaria en general, y la láctea en particular, están en constante evolución, algo imprescindible para poder hacer frente a todas las demandas del consumidor. La población cada vez requiere alimentos más saludables y sostenibles por lo que la industria tiene que hacer frente a estas peticiones, o diseñando nuevos alimentos o reformulando alimentos tradicionales.

La innovación en la industria alimentaria comprende la aplicación de los avances tecnológicos (tecnologías verdes) y la incorporación de nuevos alimentos (chía, quinoa, amaranto), entre otros aspectos, como respuesta a los cambios culturales, demográficos, sociales y saludables que afectan, de forma directa o indirecta, a todas las etapas de la cadena alimentaria: desde la producción y cosecha hasta el procesamiento, elaboración y distribución. Estos avances pueden centrarse en áreas específicas como la formulación de productos, adaptándolos a las necesidades alimentarias (alimentos para celiacos, sin lactosa, etc.) que demanda el consumidor (Earle, 1997; Senyange et al., 2025). Por tanto, las

estrategias innovadoras deben abordar el sistema alimentario en su conjunto, integrando aspectos tecnológicos, nutritivos, sociales y medioambientales. El objetivo principal es ofrecer alimentos que cumplan con las exigencias nutricionales, personales y sociales de todos los colectivos, promoviendo la sostenibilidad y la eficiencia en todo el sistema (Earle, 1997, Guiné et al., 2020), resumiendo en la estrategia de desarrollo e innovación denominada Alimentos 5S (alimentos **S**anos, **S**eguros, **S**abrosos, **S**ostenibles y **S**ocialmente aceptados)

Algunos de los puntos más destacables o hitos en la innovación alimentaria en los que se ha trabajado y se sigue trabajando en los últimos años son los siguientes:

- Desarrollo de nuevos ingredientes

Se ha pasado de materiales básicos como carbohidratos, proteínas y grasas a ingredientes más sofisticados como sustitutos de grasa, edulcorantes bajos en calorías, extractos ricos en fibra dietética, etc… Y se ha experimentado un retorno a productos naturales y a la obtención de una etiqueta “limpia” (**clean label**) para los alimentos, prescindiendo del uso de aditivos y de coadyuvantes sintéticos en la elaboración de alimentos (Lin y Wang, 2023; Nabeshima et al., 2024).

- Innovaciones en procesos de fabricación

Se han introducidos métodos mecánicos mejorados y principios de ingeniería de procesos para diseñar alimentos con estructuras, propiedades sensoriales y valores nutricionales adecuados (Earle, 1997; Bu et al., 2025; Haixi et al 2025).

- Mejoras en la conservación de alimentos

Se han introducido nuevos métodos para conseguir una mayor durabilidad de los productos elaborados, desde técnicas como la aplicación de la luz pulsada e irradiación, así como la aplicación de campos eléctricos, altas presiones o la aplicación de plasma frío, a técnicas de conservación a posteriori más innovadoras como los llamados envases inteligentes, la aplicación de atmósferas modificadas,

o envases activos. Sin embargo, entre las más actuales destacan los recubrimientos comestibles o el uso de bioconservantes. Todo con la finalidad de prolongar la vida útil de los alimentos sin alterar sabor, nutrientes ni seguridad en el producto a la par que reduce el impacto ambiental (da Silva et al., 2025; Koshani et al., 2025; Milanezzi et al 2025).

- Alimentos funcionales

Como se ha comentado anteriormente, la población demanda alimentos más saludables y sostenibles, que además ayuden a prevenir enfermedades, mantener un peso óptimo, aportar energía y aliviar el estrés (metabólico y psíquico), y todo ello está impulsando una fuerte innovación en la industria alimentaria. Con el fin de responder a estas necesidades, desde la década de los 80 del siglo XX, ha surgido una solución integral: la producción de alimentos funcionales. Los alimentos funcionales van más allá de la nutrición básica, ofreciendo beneficios específicos para la salud y contribuyendo significativamente a los objetivos de bienestar físico y mental de los consumidores (Clodoveo, 2022; Hammoudi-Halat et al., 2023). Uno de los principales métodos de producción de alimentos funcionales consiste en la incorporación de compuestos bioactivos con propiedades saludables. Existen una gran variedad de compuestos bioactivos añadidos a los alimentos tradicionales para convertirlos así en alimentos funcionales. Entre ellos figuran proteínas, probióticos, prebióticos, ácidos grasos esenciales, vitaminas, minerales, antioxidantes y fibra dietética. Estos compuestos bioactivos no solo aportan valor nutricional básico, sino que, además, actúan a nivel celular y molecular, modulando funciones fisiológicas (Cloninger et al., 2019, Guiné et al., 2020). La mayoría de estos compuestos bioactivos proceden de frutas y vegetales, pero en la actualidad, para contribuir a la sostenibilidad de la producción alimentaria, se está tratando de obtener dichos compuestos bioactivos a partir de coproductos o de productos intermedios de la industria agroalimentaria. Lo que contribuye a la reducción del desperdicio alimentario y a la valorización de los coproductos, es decir, a los objetivos de desarrollo sostenible de la Agenda de 2030, como, por

ejemplo, ODS nº11 sobre ciudades más sostenibles, nº 12 sobre producción y consumo responsables o el nº13 sobre la acción sobre el clima.

1.3.2. Productos lácteos funcionales

Los productos lácteos, gracias a su alto valor nutricional y a la gran variedad de beneficios que aportan a la salud, ocupan un lugar fundamental en la alimentación diaria. Es cierto que, a esto, hay que sumarle la amplia versatilidad de la matriz láctea, lo que facilita a la industria alimentaria el proceso de conseguir productos lácteos funcionales (Verruck et al., 2019). La existencia de estos alimentos, aportan múltiples beneficios a la salud, tanto fisiológicos como psicológicos lo que lleva a reforzar entre otros, al sistema inmunitario y contrarrestando con ello distintas patologías (de Morais, 2016). Existe un gran abanico de productos lácteos, en cuanto al tipo de matriz se refiere, esta puede ser líquida, como la leche o similares, semisólida, como el yogurt, sólida, como el queso, o espumas como los mouses o los helados. Pero cabe destacar que, entre todos los tipos de lácteos, destacan por su aceptabilidad por los consumidores de los yogures, el queso, los helados y algunos postres lácteos. De ahí que sean estos productos a los que se trata de funcionalizar, adicionando compuestos bioactivos (pondría algunos). Desde el punto de vista tecnológico, la elaboración de los productos lácteos funcionales se basa en la adición directa y la encapsulación (Villamil et al., 2020). A pesar de que la adición directa es la más sencilla, la encapsulación destaca por ser un método que altera en la menor medida posible, las características fisicoquímicas, texturales y sensoriales (Robertson et al., 2016).

1.3.2.1. Yogurt funcional

El yogurt es el producto lácteo más consumido, el cual se elabora a través de la la fermentación de la leche por *Lactobacillus bulgaricus* y *Streptococcus thermophilus*. Principalmente, los yogures son de todos los productos lácteos, el más versátil tecnológicamente hablando. Esto facilita la incorporación, a esta matriz láctea, de ingredientes bioactivos muy diversos, con características

funcionales, las cuales proporcionan una serie de características como por ejemplo una mejora del valor nutricional.

Actualmente, existen numerosos estudios sobre la funcionalización de los yogures, los cuales han sido enriquecidos con productos alimentarios intermedios obtenidos de coproductos alimentarios, contribuyendo de esta forma al desarrollo de alimentos innovadores más saludables y a reducir el desperdicio alimentario.

Los coproductos del procesamiento de frutas y verduras destacan por ser ricos en fibra dietética, compuestos bioactivos y minerales entre otros nutrientes. Como se muestra en la tabla 2, los extractos de frutas o vegetales cuenta con un propósito diferente en cuanto a su adición al yogurt, por ejemplo, la adición de la piel de la fruta de dragón (*Selenicereus undatus*) (0%, 25%, 35% y 45%) da lugar a un yogurt helado con un alto contenido en fibra y actividad antioxidante (Analianasari y Apriyani, 2018). También se observa que la harina de lenteja puede tener diferentes aplicaciones tecnológicas en este producto lácteo, como, por ejemplo, es fuente de proteínas (1%, 2%, 3% y 4%), disminuye la sinéresis (4%) del producto (Benmeziane et al., 2021) o incrementa el contenido de minerales de los yogures (Ul Haq et al., 2019). Lo mismo ocurre con la chía, por ejemplo, su mucílago puede utilizarse para sustituir parte de la grasa del alimento (0%, 2,5%, 5% y 7,5%) dando lugar así a un yogurt con un elevado contenido en fibra (Ribes et al., 2021). En cambio, adicionar las semillas de chía conlleva un incremento del contenido en proteínas, fibra dietética y minerales y una mejora del perfil de ácidos grasos (Eker y Karakaya, 2020; Kowaleski et al., 2020).

Tabla 2. Incorporación de compuestos bioactivos de frutas, vegetales y sus coproductos en yogures.

Extracto adicionado (fruta/vegetal)	Concentración (%)	Efecto	Referencias
Piel de fruta de dragón	0; 25; 35; 45	Alto contenido en fibra y actividad antioxidante	Analianasari y Apriyani (2018)
Extracto de hierbas silvestres	0; 0,25; 0,5; 0,75; 1	Elevada actividad antioxidante y valor nutricional	Dabija et al. (2018)
Mucílago de chía	0; 2,5; 5; 7,5	Aumento del contenido en fibra y mejora del valor nutricional	Ribes et al. (2021)
Harina de lenteja	0; 1; 2; 3; 4	Incremento en el contenido de minerales y proteínas	Ul Haq et al. (2019)
Salvado de arroz	0; 1; 2; 3	Mejor estabilidad, más compacto y poroso después de la adición	Wu et al. (2023)
Rosa mosqueta	0; 5; 10; 15; 20	Fuente de fitonutrientes (fenólicos, azúcar natural), mejora de la actividad antioxidante	Sahingil y Hayaloglu (2022)
Soja y quinoa	Q:S = 10:0; 8:2; 6:4; 4:6; 2:8; 0:10	Mejora de las propiedades antioxidantes	Huang et al. (2022)
Harina de *Elaeagnus angustifolia L.*	0; 1; 2	Aumento del contenido fenólico y de la actividad antioxidante	Öztürk et al. (2018)
Linaza	0; 3	Mejora de la viscosidad y disminución de la sinéresis	Basiri et al. (2022)
Semillas de chía y fresas	C: 0; 6; 14; 6; 14; 10 F: 8; 8; 12; 12; 10	Aumento del contenido de proteínas, lípidos, fibra dietética, ácidos grasos poliinsaturados (ω-3) y minerales	Kowaleski et al. (2020)
Semillas de chía	0; 2; 3; 4	Incremento del contenido de proteínas y mejora del perfil de ácidos grasos	Eker y Karakaya (2020)
Harina de lenteja	0; 4	Disminución de la sinéresis	Benmeziane et al. (2021)
Extracto de zanahoria	0; 2,5; 5	Alto contenido en β-carotenos y aumento de la actividad antioxidante	Sereglj et al. (2021)
Harina de *Pilosocereus gounellei*	0; 1; 2	Elevado contenido en minerales, compuestos fenólicos y flavonoides y mejor actividad antioxidante	Dantas et al. (2022)
Arroz y extracto de avena	Sustitución del 50%	Fuente de ω-6 y ω-9	Campos et al. (2018)
Extracto de semillas de chía	0; 0,05; 0,1	Menor tiempo de fermentación y aumento de bacterias lácticas, mejora de las propiedades fisicoquímicas y de la actividad antioxidante	Kwon et al. (2019)
Plátano y piel de plátano	0; 0,2; 0,5; 1	Alto contenido de fibra	Safdari et al. (2021)

1.3.2.2. Quesos funcionales

Actualmente se conoce que el consumo de queso está estrechamente relacionado con diversos beneficios para la salud, como mejorar la función inmunológica, la reducción del riesgo cardiovascular o la prevención de la pérdida de masa ósea (Gebreyowhans et al., 2019; Verruck et al., 2019). La industria alimentaria, para satisfacer las necesidades actuales de los consumidores, busca reducir los aditivos sintéticos y sustituirlos por ingredientes naturales con funciones similares. Por otro lado, la estructura fisicoquímica del queso facilita la incorporación de ingredientes bioactivos que dan lugar al desarrollo de alimentos funcionales, saludables y sostenibles. Además, estos ingredientes no solo enriquecen el perfil nutricional y funcional, también mejora el valor biológico y las características sensoriales (Farahat et al., 2021).

En respuesta a esta demanda de alimentos más saludables y funcionales, existen numerosos estudios en los que se ha adicionado un extracto natural a un tipo de queso con el objetivo de obtener un alimento enriquecido (tabla 3). Por ejemplo, la adición de pasta de brócoli (5%, 10% y 15%) dio lugar a un queso con menor contenido de grasa, lo que mejora el perfil nutricional. Además, la adición de brócoli también aumentó la cantidad de compuestos fenólicos, lo que a su vez incrementó la actividad antioxidante (Abd El-Montaleb et al., 2022). Cabe destacar que existen otros extractos naturales que también elevan el contenido en fenoles y la actividad antioxidante como el polvo de espinacas (El-Sayed, 2020), el zumo de tomate (Mehanna et al., 2017), o la pasta de pimientos rojos dulces (Atwaa et al., 2020) entre otros.

Existen otros estudios donde se pueden apreciar cambios en la composición nutricional del queso, el polvo de espirulina incrementó el contenido en proteínas, hidratos de carbono y fibra (Gaber Mohamed et al., 2020). La adición de setas mejoró el perfil nutricional (Petrović et al., 2015). Por otro lado, la mezcla de una serie de verduras (champiñones, eneldo, puerro, perejil, apio, guisantes, calabaza, patatas y zanahorias) para el desarrollo de queso fundido, aumento el contenido en vitaminas, minerales, aminoácidos, ácidos grasos poliinsaturados y

compuestos fenólicos totales (Lucera et al., 2018; Farahat et al., 2021; El-Loly et al., 2022).

Tabla 3. Incorporación de compuestos bioactivos de frutas, vegetales y sus coproductos en diferentes tipos de quesos.

Producto lácteo	Extracto adicionado (fruta/vegetal)	Concentración (%)	Efecto	Referencias
Queso probiótico	Pasta de brócoli (*Brassica 35lerácea*)	5; 10; 15	Disminución de grasas y proteínas. Aumento del contenido en fenoles, actividad antioxidante y vitaminas	Abd El-Montaleb et al. (2022)
Queso fresco	Polvo de espinacas (*Spinacia oleracea*)	0,5; 1; 1,5; 2	Aumento del contenido en fibra, minerales, fenoles totales y antioxidantes. Baja aceptabilidad sensorial	El-Sayed (2020)
Queso fundido	Polvo de espirulina (*Spirulina máxima*)	1; 2; 3	Incremento del contenido en proteínas, HC y fibra	Gaber Mohamed et al. (2020)
Queso fresco	Extracto de tomate (polvo microencapsulado)	0,5 - 2	Aumento del contenido en licopeno. Mayor puntuación en la evaluación sensorial	Jeong et al. (2017)
Queso fundido	Zumo de tomate	10; 20; 30	Incremento del contenido en potasio, hierro, licopeno y compuestos fenólicos	Mehanna et al. (2017)
Queso fundido	Mezcla de verduras (champiñones, eneldo, puerro, perejil, apio, guisantes, calabaza, patatas y zanahorias)	2,5; 5; 7,5; 10	Aumento del contenido en aminoácidos, ácidos grasos poliinsaturados, polifenoles totales y actividad de eliminación de radicales. Incremento de vitaminas (A, C, complejo B) y minerales (Mg, Se y Fe)	El-Loly et al. (2022)
Queso fundido	Pasta de pimiento rojo dulce	10; 20	Incremento de sólidos totales fibra, proteínas, cenizas, grasa, pH, potasio, contenido de fenoles totales	Atwaa et al. (2020)

Queso crema	Setas (*Agrocybe aegerita*)	3	Mejora del valor nutricional, sabor y olor	Petrović et al. (2015)
Queso fundido	Setas frescas y secas	0; 5; 10; 15 (frescas)/ 1; 1,5; 2 (secas)	Disminución de bacterias formadoras de esporas. Incremento de humedad, cenizas, minerales, proteínas y de bacterias lipolíticas y proteolíticas. Mejora significativa de la textura	Khider (2017)
Queso fundido	Setas secas, patata, calabaza, zanahoria, guisante, apio, puerro, eneldo y perejil	2,5; 5; 7,5; 10	Aumento de la calidad nutricional (mayor contenido en materia seca, proteínas, fibra y carbohidratos)	Farahat et al. (2021)
Queso untable	Harinas de orujo de uva, piel de tomate, brócoli, salvado de maíz y alcachofas	5	Incremento del contenido total en fenoles y flavonoides	Lucera et al. (2018)

1.3.2.3. Helados y postres lácteos

El helado consiste en un producto lácteo, en el cual el 80% de su formulación es leche y un 20% son ingredientes grasos no lácteos como edulcorantes, emulsionantes, agua, huevos, estabilizantes, colorantes y aromatizantes entre otros (Loffredi et al., 2021; Chuck-Hernandez et al., 2022). Cuenta con una matriz láctea compleja desde un punto de vista fisicoquímico, además es un producto lácteo muy extendido por todo el mundo a pesar de tener un alto contenido en azúcares y grasa, lo que podría perjudicar la salud con un consumo excesivo (Krystyjan et al., 2015).

En referencia a los postres lácteos (excluidos yogures y helados), existe una situación similar. Son productos con matrices complejas que incluyen como ingredientes principales leche, azúcar, almidón, hidrocoloides, colorantes y aromatizantes que faciliten la aceptabilidad de los consumidores.

Por ello, una opción muy viable para garantizar al consumidor el cumplimiento de sus demandas actuales sobre alimentos más saludables y funcionales, sería

funcionalizar y/o mejorar estos alimentos, reduciendo y/o sustituyendo estos ingredientes o añadiendo otros ingredientes que aporten compuestos bioactivos que puedan ayudar a:

- Reducir adecuadamente estos altos niveles de grasa y azúcares.
- Mejorar las propiedades fisicoquímicas y tecnológicas de los helados.

En términos generales, los helados y postres disponibles en el mercado suelen presentar bajos niveles de vitaminas, antioxidantes naturales como carotenoides, ácidos fenólicos y flavonoides, así como una limitada presencia de pigmentos bioactivos. En este contexto, tanto las frutas como las verduras y sus respectivos productos alimentarios intermedios se postulan como ingredientes prometedores para mejorar las propiedades fisicoquímicas y sensoriales de los helados, aportando dulzor, color, aroma y sabor (Salehi, 2020), además de enriquecer su perfil nutricional mediante la incorporación de compuestos bioactivos de interés funcional. Como consecuencia de ello, diversos estudios han explorado la formulación de helados enriquecidos con frutas y hortalizas, valorando su potencial para el desarrollo de alimentos más saludables y atractivos para el consumidor.

Singh et al. (2022) sustituyeron sólidos lácteos de los postres por pasta de dátil al 50% y 70% aumentando de esta forma el contenido en minerales, sobre todo zinc y potasio, el contenido fenólico, el contenido en ácido ascórbico y la actividad antioxidante. También se adicionó a un helado, pasta de cereza cornelina (*Cornus mas*) a concentraciones del 5%, 10% y 15% (tabla 4) incrementando de esta forma el contenido de vitaminas y flavonoides y contribuyendo a una mejora de la actividad antioxidante (Topdaş et al., 2017). Otra forma de aumentar el contenido en vitaminas, además de incrementar los minerales como el potasio, el hierro y el zinc y a su vez mejorar el perfil de compuestos bioactivos del helado es mediante la adición de zumo de bayas (3%, 6% y 10%) como se muestra en el estudio de Naeem et al. (Naeem et al., 2019). Otro ejemplo sería utilizar harina tanto de pulpa como de piel de plátano (1% y 2%) para incrementar el contenido en fibra dietética y

mejorar el perfil de minerales (Yangilar, 2015). El kiwi o sus coproductos, serían otra buena opción, ya que su adición ha demostrado un incremento en el contenido de polifenoles, lo que mejora la actividad antioxidante y un aumento de la vitamina C (Sun-Waterhouse et al., 2013).

Tabla 4. Incorporación de compuestos bioactivos de frutas, vegetales y sus coproductos en helados y postres lácteos.

Extracto adicionado (fruta/vegetal)	Concentración (%)	Efecto	Referencias
Fibras de manzana, avena y trigo	2; 4	Mejora de la calidad nutricional y aspectos fisiológicos	Soukoulis et al. (2009)
Extracto de granada o extracto de semilla de uva	0,4	Aumento del contenido total de fenoles y de la actividad antioxidante	Sagdic et al. (2012)
Kiwi	49	Incremento del contenido en polifenoles y vitamina C	Sun-Waterhouse et al. (2013)
Coproductos de cáscara, bagazo y semillas de naranja	1; 1,5	Disminución de la grasa en un 50%. Aumento del contenido en fibra y de los valores de dureza, gomosidad y elasticidad	Crizel et al. (2014)
Puré de caqui	8; 16; 24; 32; 40	Mayor contenido en minerales y mejora de las propiedades bioactivas	Karaman et al. (2014)
Harina de pulpa y piel de plátano	1; 2	Incremento del contenido de fibra y mejora del perfil de minerales	Yangilar (2015)
Kumquat (*Fortunella margarita*)	5; 10; 15	Aumento de la acidez y del contenido en vitamina C	Çakmakçi et al. (2016)
Pasta de cereza cornelina (*Cornus mas L.*)	5; 10; 15	Mejor actividad antioxidante y aumento del contenido en vitaminas y flavonoides	Topdaş et al. (2017)
Fibra de manzana, naranja, avena, trigo y bambú	2	Reducción de grasa y aumento de la supervivencia de las bacterias probióticas	Akalin et al. (2018)
Zumo de caña de azúcar	20; 40; 60	Mejora de la actividad antioxidante e inhibición de la	Ullah et al. (2015)

		oxidación de ácidos grasos insaturados	
Fruto de la palmera pindo (*Butiaodorata*)	30; 40; 50	Aumento de carotenoides y flavonoides	Dos Santos Cruxen et al. (2017)
Zumo de bayas doradas	3; 6; 10	Mejor perfil de compuestos bioactivos. Aumento de la actividad antioxidante y del contenido en vitamina C y minerales (Fe, K y Zn)	Naeem et al. (2019)
Endrinas	5; 10; 15	Incremento de potasio, manganeso, y de la actividad antioxidante	Ürkek et al. (2019)
Tallo de *Colocasia esculenta*	6	Aumento de la actividad antioxidante y alto contenido en vitamina C y E y minerales	Asaduddin et al. (2021)
Pulpa de fruta de Nabq (*Ziziphus spina-christi L.*)	25; 50; 75; 100	Alto contenido en compuestos fenólicos y mayor actividad antioxidante	Tawfek et al. (2022)

1.4. LA DIGESTIÓN "*IN VITRO*" Y SU FUNCIÓN EN LA MATRIZ ALIMENTARIA

La digestión *in vitro* es un modelo experimental utilizado para estudiar el comportamiento de los compuestos bioactivos durante la digestión gastrointestinal. Su principal objetivo es simular, de forma controlada y reproducible, los procesos fisiológicos que ocurren en la boca, el estómago y el intestino delgado, permitiendo evaluar la bioaccesibilidad, la cual hace referencia a la fracción de un nutriente o compuesto bioactivo que queda disponible para ser absorbido, así como estimar la proporción que permanece ligada a la matriz alimentaria y podría llegar al colon para ser metabolizada por la microbiota intestinal.

En la última década se han desarrollado protocolos estandarizados, siendo el modelo estático INFOGEST uno de los más aceptados a nivel internacional. Este método define condiciones replicables de pH, concentraciones enzimáticas, tiempos de incubación y composición de fluidos digestivos, permitiendo comparar resultados entre diferentes estudios y matrices alimentarias (Minekus et al., 2014; Brodkorb et al., 2019). Además, varios investigadores han llevado a cabo diversos

estudios para comparar matrices alimentarias distintas y observar cómo estas matrices modifican la estabilidad y liberación de polifenoles. Un ejemplo bastante destacado sería, Lucas-González et al. (2023), donde evaluaron diferentes matrices vegetales (tomate, pimiento, paté, entre otros) sometidas a una digestión *in vitro* siguiendo el protocolo INFOGEST, concluyendo que la bioaccesibilidad de polifenoles variaba entre ~5-38 % según matriz y procesamiento.

La digestión *in vitro* es especialmente relevante para evaluar alimentos funcionales, ya que el contenido total de compuestos bioactivos en un alimento no refleja necesariamente su fracción biodisponible. Durante el proceso digestivo, los compuestos fenólicos y otros fitoquímicos pueden:

- Liberarse de estructuras celulares, complejos macromoleculares o uniones covalentes.
- Transformarse en moléculas más simples con mayor solubilidad.
- O bien, degradarse por condiciones adversas (pH, enzimas, sales biliares, oxidación).

La matriz alimentaria desempeña un papel clave en estos procesos. La presencia de proteínas, lípidos, fibra dietética y azúcares puede proteger ciertos compuestos, facilitar su liberación o, por el contrario, favorecer su degradación o su retención en el alimento. Se ha demostrado que la afinidad de los polifenoles por proteínas (como las lácteas), la interacción con grasas y la estructura física del alimento (líquida, semisólida o sólida) influyen de manera directa en su estabilidad y bioaccesibilidad (Mandalari et al., 2016; Tarko y Duda-Chodak, 2020).

Asimismo, la digestión *in vitro* permite estimar el índice de fracción colónica, que representa la proporción de compuestos fenólicos que no se liberan ni absorben en el intestino delgado y alcanzan el colon. Allí, la microbiota puede transformarlos en metabolitos bioactivos que contribuyen a efectos beneficiosos para la salud, como la modulación de la inflamación, la actividad antioxidante o la mejora de la barrera intestinal (Cardona et al., 2013; Hao et al., 2021; Kasprzak et al., 2021).

Por tanto, la aplicación de modelos de digestión *in vitro* es esencial para comprender la funcionalidad real de los alimentos y predecir la fracción potencialmente biodisponible y el índice de fracción colónica. Estos estudios

proporcionan una visión más precisa del impacto nutricional y funcional de los compuestos bioactivos, especialmente en el diseño y desarrollo de alimentos reformulados o enriquecidos con ingredientes funcionales.

CAPÍTULO 2. OBJETIVOS



2. OBJETIVOS

2.1. OBJETIVO GENERAL

El objetivo general de esta Tesis Doctoral es aportar datos científicos que permitan a las industrias comercializadoras de dátiles frescos aprovechar al máximo la cosecha de dátiles, no solo mediante la comercialización de dátil fresco, sino mediante la valorización de los coproductos generados, obteniendo ingredientes de alta calidad nutritiva y sanitaria, lo suficientemente estables para poder ser ofrecidos a la industria alimentaria (productos alimentarios intermedios), específicamente la industria láctea, para el desarrollo de productos lácteos más saludables y sostenibles.

2.2. OBJETIVOS ESPECÍFICOS

Para alcanzar este objetivo general se proponen los siguientes objetivos específicos:

I. Comparar las diferentes variedades de dátiles comerciales procedentes de las palmeras datileras ilicitanas en función de sus características morfológicas y fisicoquímicas, sus propiedades tecnofuncionales, su composición proximal, además de su contenido en minerales, polifenoles, antioxidantes y ácidos orgánicos y azúcares.

II. Desarrollar y optimizar un proceso tecnológico que permita obtener productos alimentarios intermedios estables a partir de los coproductos generados de la comercialización del dátil fresco ilicitano.

III. Caracterizar (composición química, propiedades fisicoquímicas, propiedades tecnofuncionales y actividad biológica) dichos productos alimentarios intermedios y seleccionar las matrices lácteas más adecuadas para su incorporación.

IV. Desarrollar productos lácteos fortificados con los productos alimentarios intermedios seleccionados y evaluar su influencia sobre la calidad de los mismos fortificados.

V. Evaluar la interacción de los compuestos bioactivos de los productos alimentarios intermedios con la matriz láctea durante un proceso de digestión *in vitro*.

CAPÍTULO 3.
MATERIALES Y MÉTODOS



3. MATERIALES Y MÉTODOS

En la figura 10, se presenta un resumen del diseño experimental, así como de los materiales y métodos utilizados, para dar una visión general del trabajo llevado a cabo para alcanzar los objetivos anteriormente planteados. La metodología completa y detallada, aparece en las correspondientes publicaciones.

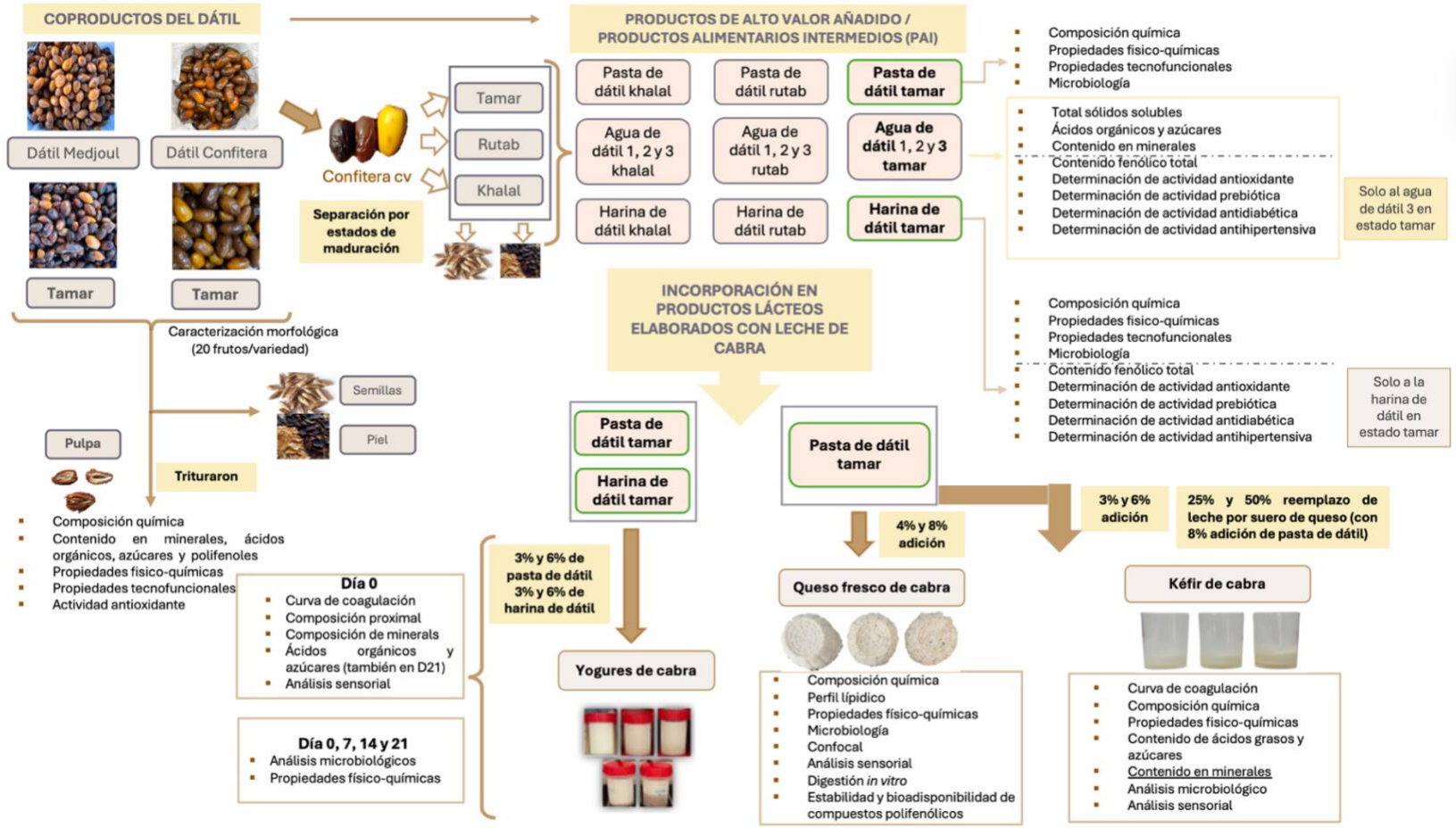


Figura 10. Esquema general del desarrollo experimental y las propiedades evaluadas

3.1. CARACTERIZACIÓN DE LOS DÁTILES FRESCOS DEL PALMERAL DE ELCHE

Desde un punto de vista comercial, el cultivar Medjoul es el predominante en el Palmeral de Elche, pero hay un cultivar autóctono, Confitera, que está últimamente atrayendo la atención de los productores de dátil.

3.1.1. Ingredientes

Se recolectaron de forma manual aproximadamente 7000 frutos en estado tamar entre octubre del 2021 y febrero de 2022 (procedentes de los cultivares Medjoul y Confitera), por personal especializado en el Palmeral de Elche (Elche, Alicante, España) y fueron transportados en condiciones de refrigeración al laboratorio del grupo de Innovación en Productos Alimentarios (IPOA) del Instituto de Investigación en Innovación Agroalimentaria y Agroambiental (CIAGRO) de la Universidad Miguel Hernández de Elche (Orihuela, Alicante, España). De entre esos 14000 frutos, finalmente se usaron 45 Kg de cada cultivar, los cuales se conservaron en refrigeración (4 ºC).

3.1.2. Caracterización morfológica de los dátiles frescos

Se midió el peso, la longitud y la anchura de 20 frutos de dátil elegidos al azar de cada cultivar (Medjoul y Confitera cv). Para ello, se separaron manualmente de los frutos seleccionados, la semilla de la pulpa y la piel, se registraron los pesos de cada una de estas tres fracciones (piel, pulpa y semillas) usando una balanza analítica PLI-360-3M (Kern & Sohn, Balingerm Alemania), la longitud y la anchura se determinaron utilizando un calibre Vernier. Los resultados se expresaron como el valor medio de cada medida con la desviación estándar de cada uno de los parámetros para ambos cultivares.

3.1.3. Propiedades físicoquímicas de los dátiles frescos

3.1.3.1. pH

El pH se determinó usando un pH-metro GLP21 (Crison, Barcelona, España) a una suspensión de 0,5 g de muestra en 50 mL de agua desionizada, homogeneizada durante 2 min. Las medidas se realizaron por triplicado para cada uno de los lotes analizados.

3.1.3.2. Medida instrumental del color

Para la evaluación de las propiedades de color de los dátiles frescos se utilizó un espectrofotómetro CM-700 (Konica Minolta Camera Co., Osaka, Japón). Para ello se seleccionó el espacio de color CIEL*a*b*, y se obtuvieron las coordenadas colorimétricas, luminosidad (L*), coordenada rojo/verde (a*) y coordenada amarillo/azul (b*). A partir de dichas coordenadas CIEL*a*b*, se calcularon las magnitudes psicofísicas croma (C*) y tono (h*) así como las diferencias de color entre ambas variedades de dátil (ΔE*) utilizando las ecuaciones 1, 2 y 3, respectivamente (Ec. 1, Ec. 2 y Ec. 3).

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad \text{(Ec. 1)}$$

$$h^* = tan^{-1}\frac{b^*}{a^*} \qquad \text{(Ec. 2)}$$

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad \text{(Ec. 3)}$$

Se trabajó con un observador de 10º y un iluminante D65, en modo SCI. También se obtuvo el espectro de reflexión de las muestras entre 360 y 740 nm, correspondiente al espectro visible. Se usó un cristal de baja reflectancia (Minolta CR-A51/1829-752)

entre las muestras y el equipo, para evitar interferencias en la medida del color. Se realizaron 18 medidas para cada cultivar a una temperatura aproximada de 25 ºC.

3.1.4. Propiedades tecnofuncionales de los dátiles frescos

3.1.4.1. Capacidad de retención de agua (CRA) y capacidad de retención de aceite (CRO)

Los análisis de la CRA y CRO se llevaron a cabo siguiendo el método de López-Marcos et al. (2015). Para ello, se pesaron en tubos de centrífuga 0,500±0,005 g de una mezcla homogeneizada de dátiles de cada cultivar y se le adicionó 10 mL de agua o aceite de girasol (para CRA y CRO, respectivamente). Los tubos se mantuvieron durante 18 h a temperatura ambiente (25 ºC). Tras este periodo de tiempo, se centrifugaron (Nahita 2653, Alicante, España) a 1200 g durante 20 minutos. Los resultados fueron expresados como g de agua o aceite retenido/g de muestra. Las determinaciones se realizaron por triplicado para cada una de las muestras.

3.1.4.2. Capacidad de hinchamiento (SWC)

La capacidad de hinchamiento de las muestras se realizó mediante la metodología descrita por López-Marcos et al. (2015). Para la determinación de SWC, se pesaron 0,200±0,005 g de una mezcla homogeneizada de dátiles de cada cultivar. Se midió el volumen ocupado por las muestras antes y después de la adición de 5 mL de agua ultrapura. La mezcla se agitó para eliminar las burbujas de aire atrapadas y se dejó durante 24 h a temperatura ambiente. Tras este periodo, se midió el volumen (mL) ocupado por las muestras de dátiles hidratadas y los resultados se expresaron como mL de agua/g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los cultivares.

3.1.5. Composición química de los dátiles frescos

El contenido de cenizas, proteínas, grasa, fibra dietética total (FDT) y humedad para las dos variedades de dátil fresco estudiadas en la presente Tesis (Medjoul y Confitera cv), se determinaron siguiendo los métodos oficiales de análisis (AOAC, 2010). El contenido total de azúcares se estimó por diferencia, de la suma de los otros componentes (cenizas, proteína, grasa, FDT y humedad) hasta el total (100%).

3.1.5.1. Cenizas

El contenido de cenizas se determinó por el método 923.023 de la AOAC (2010). Se trata de un método gravimétrico, en el cual las muestras llevan a condiciones de incineración (525 ºC) en una mufla modelo 12 PR/300 SERIE 8 (Fons Hobersal, Barcelona, España). Las determinaciones se realizaron por triplicado para cada uno de los cultivares y los resultados vienen expresados como g de cenizas/100 g de muestra.

3.1.5.2. Proteínas

La cantidad de proteínas se determinó mediante el método Kjeldahl, 981.10 de la AOAC (2010), con un factor de conversión de nitrógeno de 6,25. Para obtener la cantidad de proteína de forma directa, se utilizó un destilador automático Kjeltec TM 8400, (Foss Iberia, Barcelona, España) con una digestión previa en un digestor Büchi modelo 426 (Digestion Unit, Barcelona, España). Las determinaciones se realizaron por triplicado para cada cultivar y los resultados se expresaron como g de proteína/100 g de muestra.

3.1.5.3. Grasa total

La determinación de grasa total de las muestras se determinó siguiendo la metodología 991.36 descrita por la AOAC (2010). Utilizando como solvente extractante éter de petróleo en un extractor automático SOXTERM® SOX 6-place (Gerhardt GMBH

& Co. KG, Königswinter, Alemania). Los resultados se expresaron como g de grasa/100 g de muestra. Las determinaciones se realizaron por triplicado para cada cultivar.

3.1.5.4. Humedad

La humedad se determinó por el método 925.45 propuesto por la AOAC (2010). Se trata de un método gravimétrico donde los resultados vienen expresados en g de agua/100 g de muestra y la determinación se realizó por triplicado.

3.1.5.5. Fibra dietética total

La cantidad de fibra dietética total (FDT), se determinó por el método 985.29 propuesto por la AOAC (2010). Se trata de un método enzimático-gravimétrico, en el cual se utiliza un equipo de filtración CSF6 (Velp Scientifica, Italia) y los resultados se expresaron en g de FDT/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los cultivares analizados.

3.1.5.6. Contenido de ácidos orgánicos y azúcares

Para la extracción de la muestra, se adicionaron 50 mL de agua ultrapura con un 0,1 % (v/v) de ácido ortofosfórico a 1,000±0,005 g de la mezcla homogeneizada de dátiles de cada una de los cultivares estudiados (Medjoul y Confitera) (Agius et al., 2018). La mezcla se mantuvo en agitación durante 24 h. Tras este periodo de tiempo, las mezclas se homogeneizaron a 20000 rpm durante 2 min en un homogeneizador (Ultra-Turrax T25 BASIC, IKA-Werke GmbH & Co. KG, Staufen, Alemania) y se calentaron a 80 ºC en agitación constante durante 1 h. A continuación, las muestras se centrifugaron a 6500 g durante 10 minutos a 4 ºC y los sobrenadantes se filtraron a través de un filtro de 0,45 µm (Millipore Corporation, Bedford, EEUU). Las muestras (20 µL) se analizaron por cromatografía líquida de alta definición (HPLC) usando un equipo Hewlett-Packard modelo 1100 (Hewlett-Packar, Woldbronn, Alemania), según la

metodología descrita por Melgarejo-Sánchez et al. (2015). Se usó una columna Supelco (Supelcogel TM C-610H de 300 mm x 78 mm; Supelco, Bellefonte, EEUU) y la absorbancia se midió a 210 nm, utilizando un detector de diodos UV-visible G1315A. Se trabajó en condiciones de flujo isocrático, con ácido ortofosfórico (0,1 % v/v) como eluyente a una caudal de 0,5 mL/min. Los estándares de ácidos orgánicos, monosacáridos y oligosacáridos se obtuvieron de Supelco (Sigma-Aldrich, St. Louis, EEUU). Los picos se identificaron mediante comparación con el tiempo de retención de los estándares y se cuantificaron por medio del uso de las rectas de regresión obtenida con los estándares. Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los cultivares analizados.

3.1.5.7. Contenido en minerales

Para la determinación y cuantificación del contenido en minerales, previamente se liofilizaron las muestras de dátil de los cultivares analizados con un liofilizador modelo Alpha 2-4 (Martin Christ Gefriertrocknungsanlagen GmbH, Alemania). La determinación se realizó mediante espectrometría de masas con plasma acoplado inductivamente (ICP-MS-2030) (Shimadzu, Kioto, Japón). La cuantificación del contenido en minerales de las muestras se realizó tras una digestión con ácido nítrico (67% v/v) y peróxido de hidrógeno (33% v/v) mediante un sistema de microondas modelo Mars one (CEM, Carolina del Norte, EEUU). Para calibrar el ICP-MS y poder medir el contenido en minerales de las muestras, se diluyeron los compuestos estándar. El ICP-MS operó con las siguientes condiciones: el flujo de gas nebulizado fue de 0,91 L/min, la radiofrecuencia fue de 1200 W, la tensión de la lente de 1,6 V y los flujos del gas refrigerante y del gas auxiliar de 12,0 L/min y de 0,70 L/min, respectivamente. Los resultados se obtuvieron tras analizar un triplicado de dos

muestras de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de muestra.

3.1.5.8. Contenido en polifenoles

Para este análisis se realizó una extracción siguiendo la metodología descrita por Gensowsky et al. (2016). Se pesaron 2,000±0,005 g de muestra en tubos de centrífuga de polietileno con tapón. A continuación, se añadieron a las muestras 20 mL de una mezcla metanol-agua (80:20 v/v), se homogeneizó la muestra durante 3 minutos a 18000 rpm con un ultraturrax (Dispensador T 25 Basic ULTRA-TURRRAX®, IKA®, Staufen, Alemania). Tras la homogeneización, se centrifugó la mezcla durante 10 min a 8000 g y 4 ºC. El sobrenadante se depositó en un matraz de fondo plano de 100 mL y a la fase sólida se le adicionó 20 mL de una mezcla acetona-agua (70:30 v/v). La mezcla volvió a ser homogeneizada y centrifugada en las mismas condiciones descritas previamente. Los sobrenadantes se mezclaron y se llevaron a sequedad en un rotavapor (Büchi Rotavapor R-200, Büchi Ibérica, Barcelona, España). Tras esta etapa, se redisolvió en 5 mL de agua ultrapura. Para minimizar cualquier posible interferencia por el contenido de azúcares de las muestras durante el análisis por cromatografía, se utilizó un cartucho C-18 Sep-Pak (Thermo Scientific™ Cartridge HyperSep™ C18, Fisher Scientific, Madrid, España), activado previamente con 3 mL de metanol grado HPLC, 3 mL de agua ultrapura y 3 mL de ácido clorhídrico 0,01 N. Posteriormente el cartucho se lavó con 5 mL de agua ultrapura, la elución de la muestra se llevó a cabo con 3 mL de metanol grado HPLC acidificado al 0,1 g/L de ácido fórmico. Los extractos resultantes se conservaron cuidadosamente a −18 ºC hasta el análisis por HPLC. Las muestras se analizaron mediante un espectrómetro de masas triple cuádruplo HPLC-MS/MS 8050 (Shimadzu, Kioto, Japón). Las separaciones cromatográficas se realizaron con una columna Mediterranean SEA18 (10 mm L × 0,21 mm i.d., tamaño de partícula de 2,2 µm, Teknokroma, Barcelona, España) mantenida a una temperatura de 50 ºC. Las condiciones de la fuente ISE fueron: flujo de gas nebulizador de 3 L/min, flujo de gas de

secado de 10 L/min, una temperatura de la línea de desolvatación de 250 ºC y una temperatura del bloque térmico de 400 ºC. Se realizó un monitoreo de iones seleccionados (SIM) con una energía de colisión de −35 V y barridos masas completos en modo positivo entre 100 y 1000 m/z. La cuantificación se realizó por regresión lineal. Los resultados se obtuvieron tras analizar un triplicado de las muestras de cada uno de los lotes. Los resultados se expresaron como µg compuesto fenólico/g muestra.

3.1.6. Actividad antioxidante de los dátiles frescos

Para la evaluación de la actividad antioxidante de las muestras de dátiles frescos de ambos cultivares, Medjoul y Confitera, se aplicaron cuatro métodos antioxidantes para poder evaluar diferentes mecanismos antioxidantes. Las determinaciones se realizaron espectrofotométricamente con un espectrofotómetro HP 8451 (Hewlett Packard, Cambridge, Reino Unido).

3.1.6.1. Método DPPH (radical 2,2-difenil-1-picrilhidrazilo)

El ensayo DPPH se caracteriza por medir la capacidad de las muestras de atrapar el radical DPPH o donar hidrógenos. Las determinaciones se llevaron a cabo siguiendo la metodología propuesta por Brand-Williams et al. (1995). Para poder extrapolar los resultados, se realizó una recta de calibrado con Trolox. La absorbancia en la cual se midieron las muestras fue de 517 nm. Las medidas se realizaron por triplicado y los resultados se expresaron en µg de equivalentes de Trolox/g de dátiles.

3.1.6.2. Método ABTS (radical 2,2'-azino-bis-(3-etilbenzotiazolin)-6-sulfonato de amonio)

El ensayo ABTS se llevó a cabo según el método descrito por Gullón et al. (2015), por el cual se mide la capacidad de atrapar el radical catiónico $ABTS^{\bullet+}$. La

determinación se realizó por triplicado y se midió a una longitud de onda de 734 nm. Para poder extrapolar los resultados se utilizó una curva de Trolox como antioxidante de referencia. Los resultados se expresaron en mg equivalentes de Trolox/g de dátiles.

3.1.6.3. Método FRAP (por reducción del ion férrico)

Este método se basa en la medida de la transferencia de electrones. La determinación se llevó a cabo siguiendo la metodología propuesta por Oyaizu (1986), midiendo la absorbancia a 700 nm. Para su determinación se utilizó una recta de calibrado con Trolox como antioxidante referente en las mismas condiciones en las cuales se trataron las muestras. Las determinaciones se realizaron por triplicado y los resultados se expresaron como mg equivalentes de Trolox/g de dátiles.

3.1.6.4. Método FIC (por quelación de iones ferrosos)

La actividad quelante de iones ferrosos, se determinó estableciendo la inhibición de la formación del complejo Fe^{2+}-ferrozina tras añadir Fe^{2+} a las muestras mediante el método descrito por Mahdavi et al. (2017). Los valores de absorbancia se midieron en un espectrofotómetro a 562 nm y se utilizó etilendiaminotetraacético (EDTA) como patrón de referencia. Las determinaciones se realizaron por triplicado y los resultados se expresaron como µg equivalentes de EDTA/g de dátiles.

3.1.7. Análisis estadístico

Cada cultivar de los dátiles frescos se analizó por triplicado. Se realizaron veinte réplicas del análisis de las características morfológicas. Para las medidas instrumentales de color se realizaron 18 medidas para cada lote analizado. Para el resto de las propiedades analizadas (pH, propiedades tecnofuncionales, composición química y actividad antioxidante), cada lote fue analizado por triplicado. Los resultados

de la caracterización de los dátiles frescos de los cultivares Medjoul y Confitera vienen expresados como la media de los valores, más menos la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de un factor para determinar si existieron diferencias estadísticamente significativas entre las determinaciones realizadas en los distintos tipos de cultivares de dátiles frescos. El programa usado para determinar las diferencias estadísticas fue el SPSS versión 27.0 (SPSS Inc., Chicago, USA).

3.2. PROCESAMIENTO DE LOS COPRODUCTOS DEL DÁTIL Y OBTENCIÓN DE LOS PRODUCTOS DE ALTO VALOR AÑADIDO (PRODUCTOS ALIMENTARIOS INTERMEDIOS)

Una vez seleccionado el cultivar Confitera, se procedió al procesamiento de los coproductos generados de su industrialización. Los coproductos fueron proporcionados por la Cátedra Palmeral d'Elx (UMH, Elche, España), procedentes de dátiles recolectados en diferentes estados de maduración (khalal, rutab y tamar), por lo que se procesaron de forma independiente. La manipulación de los dátiles se realizó bajo correctas prácticas de manipulación de alimentos, así como de una correcta higienización de los utensilios, aparatos y de la planta piloto. La figura 11, muestra todo el procedimiento para la obtención de los diferentes productos alimentarios intermedios, que a continuación se detallan. A partir de la materia prima inicial (coproductos de dátil Confitera en tres estados de maduración diferentes) se obtuvieron tres productos de alto valor añadido (pasta de dátil, agua de dátil y harina de dátil) de los tres estados de maduración diferentes. Las pastas y las harinas de dátil obtenidas se almacenaron bajo condiciones de vacío en bolsas de plástico protegidas de la luz (las harinas se conservaron a temperatura ambiente y las pastas a -18 ºC); las aguas de dátil se mantuvieron a -18 ºC en botellas de cristal opaco.

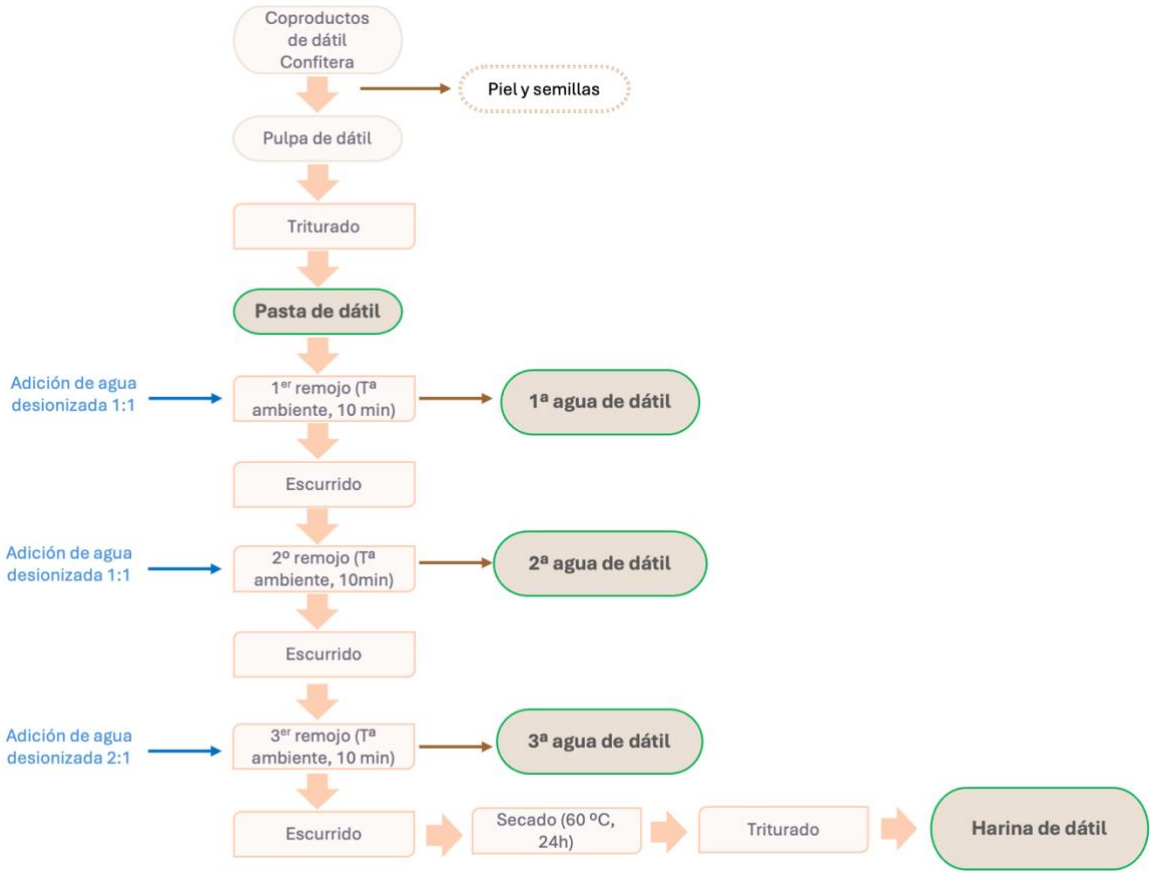


Figura 11. Diagrama de procesamiento de los coproductos de dátil y obtención de los productos de alto valor añadido (productos alimentarios intermedios).

3.2.1. Obtención de pasta de dátil

Los coproductos recibidos se separaron en lotes dependiendo del estado de maduración en el cual se encontraban (khalal, rutab y tamar) y fueron independientemente procesados. A los tres lotes se les extrajo de forma manual la semilla y la piel, las pulpas se homogeneizaron con un Homogeneizador modelo 1094 (Tekator, Höganäs, Suecia) para obtener las pastas de dátiles. En concreto fueron 3 lotes diferentes de pasta de dátil: pasta de dátil en estado khalal (KHP); pasta de dátil en estado rutab (RTP) y pasta de dátil en estado tamar (TMP). Este procesado se le realizó a cada lote por triplicado (figura 11).

3.2.2. Obtención del agua de dátil

Parte de los lotes obtenidos de pasta de dátil anteriormente mencionados (KHP, TMP y RTP), se pusieron en contacto en una proporción 1:1 con agua destilada (primer remojo) y se mantuvieron a temperatura ambiente (25 ºC) durante 10 minutos, en agitación constante. Transcurrido este tiempo, las aguas de dátil correspondientes se separaron de la parte sólida mediante prensado con paños filtrantes de algodón. Se obtuvieron así los lotes de aguas de dátil del primer remojo, en concreto: agua de dátil en estado khalal (KHA1), agua de dátil en estado rutab (RTA1) y agua de dátil en estado tamal (TMA1). A la parte sólida que resultó tras el filtrado con los paños de algodón, se le sometió al mismo proceso de remojo, obteniendo así los lotes de aguas de dátil del segundo remojo, en concreto: agua de dátil en estado khalal tras el segundo remojo (KHA2), agua de dátil en estado rutab tras el segundo remojo (RTA2) y agua de dátil en estado tamal tras el segundo remojo (TMA2). Para la obtención de la tercera agua de dátil se necesitó incorporar una mayor proporción de agua (1:2) manteniendo el resto de las condiciones de los remojos anteriores. Se obtuvieron así los lotes de aguas de dátil del tercer remojo, en concreto: agua de dátil en estado khalal tras el tercer remojo (KHA3), agua de dátil en estado rutab tras el tercer remojo (RTA3) y agua de dátil en estado tamal tras el tercer remojo (TMA3). Este procesado se le realizó a cada lote por triplicado (figura 11).

3.2.3. Obtención de la harina/polvo de dátil

A continuación, la parte sólida obtenida tras el tercer lavado y filtrado de cada lote de pasta de dátil se distribuyó en bandejas y se secó en un horno con ventilación forzada a 60 °C durante 24 h. La pasta de dátiles seca se molió y se tamizó para obtener harinas de dátiles con un tamaño específico de partícula (<0,52 mm). Se obtuvieron así tres harinas de dátil, denominadas: harina de dátiles en la etapa khalal (KHH);

harina de dátiles en la etapa rutab (RTH) y harina de dátiles en la etapa tamar (TMH). Este proceso se realizó por triplicado para cada uno de los tres lotes (figura 11).

3.3. CARACTERIZACIÓN DE LOS PRODUCTOS ALIMENTARIOS INTERMEDIOS OBTENIDOS DEL PROCESAMIENTO DE LOS COPRODUCTOS DE DÁTIL

3.3.1. Caracterización de las pastas y las harinas de dátil

3.3.1.1. Composición química de las pastas y de las harinas de dátil

Se determinó la cantidad de humedad, cenizas, grasa, proteína y fibra dietética total según el método correspondiente de la AOAC (AOAC, 2010), tal y como ya se describió en apartados anteriores 3.1.5.1; 3.1.5.2.; 3.1.5.3.; 3.1.5.4. Estas determinaciones se realizaron por triplicado en los tres lotes de pasta de dátiles (KHP, RTP y TMP) y en los tres lotes de harina de dátil (KHH, RTH y TMH). Los resultados de todos los parámetros se expresaron en g/100 g de pasta de dátil o de harina de dátil.

3.3.1.2. Determinación del contenido en azúcares y ácidos orgánicos en las pastas y en las harinas de dátil

La determinación del contenido de ácidos orgánicos y azúcares se realizó según lo descrito con anterioridad en el apartado 3.1.5.6. con modificaciones. Se pesaron 2,000±0,005 g bien de pasta de dátil o de harina de dátil y se le adicionaron 50 mL de agua ultrapura durante 24 h. El resto de las condiciones y del procedimiento fue el descrito en el apartado anteriormente mencionado (3.1.5.6.). Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los lotes analizados de pastas de dátil y de harinas de dátil.

3.3.1.3. Contenido en minerales de las pastas y de las harinas de dátil

El contenido en minerales de los tres lotes de pasta de dátil (KHP, RTP y TMP) y en los tres lotes de harina de dátil (KHH, RTH y TMH) se determinó siguiendo las mismas condiciones mencionadas con anterioridad en el apartado 3.1.5.7. A excepción de que las muestras de las harinas de dátil no precisaron de liofilización previa. Los resultados se obtuvieron tras analizar un triplicado de dos muestras de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de muestra.

3.3.1.4. Propiedades físicoquímicas de las pastas y de las harinas de dátil

3.3.1.4.1. pH

La determinación de pH se realizó en una suspensión con agua desionizada en una proporción de 1 g de muestra (pasta o harina de dátil) con 9 mL de agua desionizada, homogeneizada durante 2 min. La medida se realizó utilizando el pH-metro mencionado con anterioridad en el apartado 3.1.3.1. Las medidas se realizaron por triplicado para cada uno de los lotes analizados.

3.3.1.4.2. Medida instrumental del color

Los parámetros de color para los distintos lotes de pastas y harinas de dátil, se determinó bajo las mismas condiciones, con la misma metodología y el mismo espectrofotocolorímetro descrito en el apartado 3.1.3.2. Se realizaron 18 medidas para cada lote tanto de las pastas como de las harinas de dátil, a una temperatura aproximada de 25 ºC.

3.3.1.5. Propiedades tecnofuncionales de las pastas y de las harinas de dátil

Se midieron las mismas propiedades tecnofuncionales que en el apartado 3.1.4.2. (capacidad de retención de agua (CRA), capacidad de retención de aceite (CRO) y capacidad de hinchamiento (SWC)), bajo las mismas condiciones y con la misma metodología descrita en el mencionado apartado (3.1.4.2.). Las determinaciones se realizaron por triplicado para cada uno de los lotes tanto de pasta de dátil (KHP, RTP y TMP) como para la harina de dátil (KHH, RTH y TMH). Los resultados para la CRA y CRO fueron expresados como g de agua o aceite retenido/g de muestra y para SWC como mL de agua/g de muestra.

3.3.1.6. Análisis microbiológico de las pastas y de las harinas de dátil

Se realizó un muestreo microbiológico para las muestras de pastas y harinas de dátil con el fin de determinar la calidad higiénica de las mismas: cantidad total de bacterias mesófilas aeróbicas, mohos y levaduras, así como la cantidad de enterobacterias presentes, tanto de las pastas como de las harinas de dátil en los tres estados de maduración (KHP, RTP; TMP; KHH, RTH y TMH). Diez gramos de pasta o de harina de dátil fueron homogeneizados con 90 mL de agua de peptona al 0,1% (p/v) en un homogeneizador mecánico BagMixer interscience (France) durante 60 s. Se prepararon diluciones decimales y se sembró 1 mL por duplicado en Petrifilm (3M, Madrid, España). Para el recuento total de bacterias mesófilas aeróbicas, las muestras se sembraron en placas de recuento aeróbico y se incubaron a 37 ºC durante 48 h; en el caso del recuento de mohos y levaduras, se realizaron en placas de recuento de mohos y levaduras incubadas a 25 ºC durante 72 h. Por último, para las enterobacterias, las muestras se sembraron en placas de recuento de *Enterobacteriaceae* y se incubaron durante 24 h a 37 ºC. El recuento se realizó de forma manual de las placas que presentaron un crecimiento entre 30 y 300 unidades

formadoras de colonias (UFC) y los resultados se expresaron como logaritmo de UFC/ g de pasta o de harina.

3.3.2. Caracterización de las aguas de dátil

Cada una de las aguas de dátil obtenidas durante el procesamiento de los coproductos de cada estado de maduración y durante los tres procesos de remojo y escurrido (KHA1, RTA1, TMA1, KHA2, RTA2, TMA2, KHA3, RTA3 y TMA3) fue sometida a los siguientes análisis.

3.3.2.1. Determinación del contenido total de sólidos solubles de las aguas de dátil

La determinación del contenido total de sólidos solubles para los 9 lotes de las aguas de dátil se realizó con un refractómetro digital Milwaukee MA 871 (Milwaukee electronics, WI, USA). La determinación se realizó por triplicado para cada uno de los lotes y los resultados se expresaron como ºBrix.

3.3.2.2. Determinación de ácidos orgánicos y azúcares de las aguas de dátil

La determinación de los ácidos orgánicos y azúcares presentes en los diferentes lotes de las aguas de dátil se realizó sin una extracción previa, únicamente las muestras se centrifugaron a 6500g durante 10 minutos a 4 ºC y los sobrenadantes se filtraron a través de un filtro de 0,45 µm (Millipore Corporation, Bedford, EEUU). Tras este procedimiento, las muestras se analizaron por cromatografía líquida de alta definición (HPLC) en las condiciones y con la metodología descrita previamente en el apartado 3.1.5.6. Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada una de las aguas de dátil analizadas.

3.3.2.3. Determinación del contenido en minerales de las aguas de dátil

El contenido en minerales de los nueve lotes de las aguas de dátil se determinó siguiendo las mismas condiciones mencionadas en el apartado 3.1.5.7. Los resultados se obtuvieron tras analizar un triplicado de dos muestras de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de muestra.

3.3.3. Análisis estadístico

Los datos se analizaron utilizando el software SPSS (IBM SPSS Statistics versión 26). Se aplicó un análisis de varianza ANOVA un factor con un nivel de confianza del 95 % y se realizó la prueba de Tukey para determinar cualquier diferencia significativa ($p < 0,05$) entre las tres etapas de maduración de los dátiles Confitera (khalal, rutab y tamar). Todos los datos se presentan como valores medios ± desviaciones estándar de tres o más experimentos independientes.

3.4. EVALUACIÓN *IN VITRO* DE LAS PROPIEDADES BIOLÓGICAS DE LA HARINA Y DEL AGUA DE DÁTIL

La evaluación *in vitro* de las propiedades biológicas en las harinas y aguas de dátil únicamente se realizó de las procesadas en estado tamar, porque su composición era más apropiada para tales efectos. Las determinaciones que se le realizaron para evaluar dichas propiedades biológicas fueron: cantidad de fenoles totales, propiedades antioxidantes, propiedades antidiabéticas, propiedades antihipertensivas y propiedades prebióticas.

3.4.1. Preparación de la muestra

Para poder evaluar las propiedades biológicas de la harina de dátil, se requirió previamente una extracción siguiendo el método descrito por Hung et al. (2011) con modificaciones. A 2,000±0,005 g de harina de dátil, se le adicionaron 20 mL de etanol 80% (previamente preparado a partir de etanol absoluto), la disolución se homogeneizó en un agitador orbital (Wiggenhouse, Berlín, Alemania) a 200 rpm durante 20 min a una temperatura aproximada de 30 ºC. Tras ello la muestra se llevó a un baño de sonicación (Bandelin, Berlín, Alemania) durante 10 min y se centrifugó a 3850g en una centrífuga Hettich Universal 320R (Andreas Hettich GMBH & Co.) durante 5 min a 20 ºC. El sobrenadante se recogió en un nuevo tubo de 50 mL y sobre la parte sólida se adicionaron 10 mL de etanol al 80% y se repitió de nuevo todo el proceso. Las muestras se concentraron utilizando un rotavapor (Buchi, Flawil, Suiza) a una temperatura de 45 ºC a una presión de 100 atm, hasta reducir a 5 mL el volumen total.

3.4.2. Propiedades biológicas de la harina y agua de dátil tamar

3.4.2.1. Determinación del contenido fenólico total

El contenido fenólico total se determinó siguiendo el método propuesto por Coscueta et al. (2018), con ligeras modificaciones. Se utilizó una microplaca de 96 pocillos, se pipetearon 30 µL de cada muestra o de su dilución adecuada y se adicionaron 100 µL de reactivo Folin-Ciocalteu (20 % v/v) y 100 µL de disolución de carbonato de sodio anhidro (7,4 % p/v) en cada pocillo. También se utilizó una curva estándar de ácido gálico (AGE) con concentraciones entre 0,025 y 0,200 mg/mL para poder extrapolar los resultados. La microplaca fue incubada durante 30 min a 25 ºC en oscuridad. La mezcla resultante se midió a una absorbancia de 765 nm en un lector de detección de placas múltiple (Synergy H1, VT, EEUU) con el sofware Gen5. Todas las

mediciones se realizaron por triplicado y los resultados vienen expresados como mg de AGE/mL de muestra.

3.4.2.2. Determinación de la actividad antioxidante

La actividad antioxidante se evaluó mediante el ensayo de eliminación de 2,2-azinobis-(3-etilbenzotiazolina-6-sulfónico) (ABTS) propuesto por Gonçalves et al. (2009) con modificaciones. Para crear la curva estándar de Trolox, se pesaron 0,0125 g de Trolox y se disolvieron en 1 ml de metanol y luego se diluyó hasta un volumen final de 50 mL con agua desionizada. Las concentraciones de la curva estándar fueron desde 25 µM hasta175 µM. Para la determinación, en una microplaca de 96 pocillos, se añadieron 20 µL de Trolox o muestra y 180 µL de disolución ABTS (con una absorbancia entre 0,700- 0,720 nm) a cada pocillo. A continuación, la microplaca se incubó durante 5 minutos a 30 ºC y se midió la absorbancia a 734 nm utilizando un lector de placas de detección múltiple (Synergy H1, VT, EEUU). Todos los análisis se realizaron por triplicado y los resultados se expresaron como µM equivalentes de Trolox/mL de muestra.

3.4.2.3. Determinación de la actividad prebiótica

La determinación prebiótica se realizó tanto a la harina como al agua de dátil. Se evaluó el potencial de estos productos para promover el crecimiento y la actividad metabólica de diferentes cepas probióticas. Para el agua de dátil, al tratarse de una matriz líquida, la evaluación del potencial prebiótico se realizó a microescala utilizando el método de microplaca de 96 pocillos, que permite determinar el crecimiento celular mediante una medida de absorbancia. En el caso de la harina de dátil, al tratarse de una matriz sólida, el potencial prebiótico se evaluó a macroescala, determinando el crecimiento celular mediante el recuento de células viables, utilizando el recuento de unidades formadoras de colonias (UFC).

3.4.2.3.1. Preparación del medio de cultivo

El medio basal utilizado para evaluar el potencial bifidogénico del agua y la harina de dátil fue el caldo Man-Rogosa-Sharpe (MRS), preparado mediante la mezcla de diferentes componentes para permitir la sustitución de la fuente de carbono. La composición de este medio MRS fue (10 g/L de triptófano, 8 g/L de extracto de carne, 4 g/L de extracto de levadura, 2 g/L de hidrógenofosfato de dipotasio, 1g/L de Tween 80, 5g/L de acetato de sodio, 2 g/L de citrato de amonio tribásico, 0,2 g/L de sulfato de magnesio, 0,04 g/L de sulfato de manganeso y 20 g/L de fuente de carbono). A partir de este medio basal, se prepararon los siguientes medios:

- Caldo basal MRS sin glucosa, como control negativo.
- MRS con un 2% (p/v) de glucosa como control positivo.
- MRS con un 2% (p/v) de fructooligosacáridos (FOS) como control prebiótico positivo.
- MRS con un 2% y un 10% (v/v) de agua de dátil.
- MRS con un 2% y un 6% (v/v) de harina de dátil.

Las dos concentraciones de agua y de harina de dátil probadas, se establecieron en función del contenido total de azúcares respectivo, para aproximarse a la cantidad encontrada en el medio MRS. En el caso de las cepas de *Bifidobacterium*, todos los medios se suplementaron posteriormente con 0,5 g/L de L-cisteína-HCl esterilizado por filtración.

3.4.2.3.2. Crecimiento bacteriano

Para el ensayo en microplacas, se seleccionaron 11 cepas probióticas para su posterior cribado: *Lactobacillus acidophilus* KI, *Lacticaseibacillus paracasei* L26, *Lacticaseibacillus rhamnosus* R11, *Lacticaseibacillus casei* 01, *Lactobacillus acidophilus* La-5, *Lactiplantibacillus plantarum* 299v, *Bifidobacterium animals subespecie lactis* BB-12®, *Bifidobacterium breve* NCIMB, *Bifidobacterium animalis* Bo,

Bifidobacterium longum BG3 y *Bifidobacterium animalis* BLC. De entre esas cepas probióticas, se seleccionaron las de mejor rendimiento (mayor crecimiento) para el ensayo de microplacas, en concreto *L. casei* 01, *L. rhamnosus* R11, *B. breve* NCIMB y *B. animalis* BLC. Para cada experimento, las cepas bacterianas se reactivaron en el caldo adecuado durante 24 h. Para el crecimiento de las cepas de *Lactobacillus* se utilizó medio MRS, mientras que para el crecimiento de las cepas de *Bifidobacterium* se suplementó posteriormente el medio MRS con 0,5 g/L de L-cisteína-HCl esterilizado por filtración. Las cepas de lactobacilos se cultivaron en condiciones aeróbicas, mientras que las cepas de bifidobacterias se cultivaron en condiciones anaeróbicas (85 % N_2, 5 % H_2 y 10 % CO_2), lo que se logró utilizando una incubadora anaeróbica Whitley A35 (HEPA, Bingley, Reino Unido), después de descongelar una reserva de glicerol. Para todas las cepas, se llevó a cabo un único paso de subcultivo en condiciones de crecimiento idénticas, con un volumen final de incubación de 10 ml de MRS (suplementado con 0,05 % de cisteína para *Bifidobacterium*) y un 1 % (v/v) de inoculación celular. El recuento inicial de UFC en cada suspensión de inóculo se determinó preparando diluciones seriadas en tampón fosfato salino (PBS). Posteriormente, se sembraron 10 µl de cada dilución por triplicado en medios adecuados. Las placas de agar se incubaron a 37 ºC durante 48 h en condiciones anaeróbicas o aeróbicas para las cepas de bifidobacterias y lactobacilos, respectivamente. Tras la incubación, se realizó un recuento de UFC y los resultados se expresaron como media ± desviación estándar de UFC/ml para las suspensiones bacterianas.

3.4.2.3.3. Selección del crecimiento bacteriano mediante ensayo de microplacas

El cribado del crecimiento bacteriano de las 11 cepas probióticas mediante el ensayo de microplacas se realizó según Sousa et al. (2015), con algunas modificaciones. Cada medio preparado previamente (detallado en el apartado

3.4.2.3.1.) se inoculó con la cepa probiótica correspondiente a una concentración del 2 % (v/v), por triplicado. Posteriormente, se adicionaron 250 µl del medio de crecimiento con incorporación de los probióticos a cada pocillo de una microplaca de 96 pocillos. Para evitar la presencia de oxígeno, se añadieron 50 µl de parafina líquida esterilizada en autoclave (Merck, Alemania). El crecimiento celular se monitorizó durante 24 h midiendo la densidad óptica (DO) de los cultivos a 655 nm utilizando un lector de microplacas 680 de Bio-Rad (Hercules, CA, EEUU.) junto con el software Microplate Manager 5.2.1. Se establecieron un control negativo (utilizando MRS sin glucosa), un control de crecimiento y un control positivo (suplementando el MRS con glucosa y FOS respectivamente). Las tasas de crecimiento específicas se determinaron calculando la pendiente de la línea de tendencia y los valores de absorbancia en la fase logarítmica de las curvas de crecimiento. Se evaluó el crecimiento máximo (absorbancia máxima) para comparar los resultados de las diferentes condiciones de crecimiento.

3.4.2.3.4. Evaluación del potencial bifidogénico mediante la determinación del número de células viables

Se evaluó el potencial bifidogénico para la harina de dátil mediante la evaluación del comportamiento de crecimiento de las cuatro cepas probióticas comerciales seleccionadas (*Lacticaseibacillus rhamnosus* 11, *Lactobacillus casei* 01, *Bifidobacterium breve* NCIMB y *Bifidobacterium animalis* BLC). Se midieron sus capacidades de crecimiento y de acidificación en cada uno de los cinco medios de cultivo descritos anteriormente (apartado 3.3.2.3.1.) mediante el recuento del número de células viables y la medición de la evolución del pH, siguiendo el procedimiento descrito por Sousa et al. (2015). El metabolismo bacteriano se evaluó midiendo el pH con un pH-metro (Crison Instruments, Barcelona, España). Cada medio se inoculó con la cepa probiótica correspondiente a una concentración del 2 % (v/v). A continuación, los medios inoculados se transfirieron a tubos Eppendorf estériles de 2 ml (por

triplicado) y se incubaron a 37 ºC durante 24 h en condiciones aeróbicas y anaeróbicas para las cepas de lactobacilos y bifidobacterias, respectivamente. Para garantizar las condiciones anaeróbicas para las cepas de *Bifidobacterium*, los medios se suplementaron con 0,5 g/L de L-cisteína-HCl esterilizada por filtración y se mantuvieron en una cámara de anaerobiosis. El muestreo se realizó a las 0, 3, 6, 10 y 24 h. Se prepararon diluciones decimales en PBS en cada intervalo de muestreo y se sembraron tres veces 10 µL de cada dilución en medios adecuados. Tras la incubación, se realizó el recuento de UFC y los resultados se expresaron en UFC/mL.

3.4.2.4. Actividad antidiabética (ensayo de inhibición de α-glucosidasa)

La actividad antidiabética se evaluó midiendo la actividad inhibidora de la α-glucosidasa, siguiendo el método descrito por Kwon et al. (2008). Inicialmente, se colocaron 50 µL de muestra en cada pocillo y se le adicionaron 100 µL de disolución de α-glucosidasa (1,0 U/mL) diluida en tampón fosfato 0,1 M (pH 6,9). A continuación, la microplaca se incubó durante 10 min a 25 ºC. Tras la incubación, se adicionaron 50 µL de disolución de p-nitrofenil-α-D-glucopiranosida 5 mM (en tampón fosfato 0,1 M) a cada pocillo. Las mezclas se incubaron a 25 ºC durante 5 min y se tras este tiempo se midió la absorbancia a 405 nm utilizando un lector de placas de detección múltiple (Synergy H1, VT, EEUU). Se realizó un control negativo utilizando 50 µL de disolución tampón y un control positivo con 50 µL de acarbosa a una concentración de 10 mg/mL. Todas las mediciones se realizaron por triplicado y el porcentaje de inhibición de la α-glucosidasa se calculó utilizando la siguiente ecuación (Ec. 4):

$$\% \text{ Inhibición de α-glucosidasa} = \left(\frac{\Delta \text{Abs}_{Control} - \Delta \text{Abs}_{muestra}}{\Delta \text{Abs}_{Control}}\right) \text{x } 100 \qquad \text{(Ec. 4)}$$

3.4.2.5. Actividad antihipertensiva (ensayo de la actividad inhibidora de la enzima convertidora de angiotensina I)

El efecto antihipertensivo se evaluó mediante una prueba de actividad inhibidora de la enzima convertidora de angiotensina I (ECA), tal y como describen Sentandreu y Toldra (2006), con modificaciones. En cada pocillo se añadieron 40 µL de agua ultrapura o de disolución de ECA (42 mU/mL), y se llevó a un volumen final del pocillo de 80 µL utilizando agua ultrapura. A continuación, se añadieron 160 µL de disolución de sustrato (0,45 mM), la mezcla resultante se incubó durante 30 min a 37 ºC para permitir que se produjera la reacción enzimática. A lo largo de los 30 minutos, se midió la fluorescencia con longitudes de onda de excitación y emisión de 350 y 420 nm respectivamente, utilizando un lector de placas de detección múltiple (Synergy H1, VT, EEUU). Todas las mediciones se realizaron por triplicado y la actividad inhibidora de la ACE se calculó utilizando la siguiente ecuación (Ec. 5):

$$\% \text{ Inhibición de ACE} = ((F_{Control} - F_{Blanco}) - (F_{SPL} - F_{SPLB}))x\frac{100}{F_{Control} - F_{Blanco}} \qquad \text{(Ec. 5)}$$

3.4.3. Análisis estadístico

Los datos de esta investigación se analizaron utilizando el software SPSS versión 17.0 (SPSS; Chicago, IL, EE. UU.). Estos datos se expresaron como la media ± desviación estándar de las réplicas. Todos los experimentos se realizaron por triplicado. Se realizaron pruebas paramétricas con los datos que se encontraron que seguían una distribución normal según la prueba de Shapiro-Wilk (prueba de normalidad). Se realizó una prueba t de *Student* para muestras independientes con el fin de realizar una comparación estadística entre el agua de dátil y la harina de dátil. Las diferencias estadísticas se consideraron significativas con $p < 0,05$.

3.5. APLICACIÓN DE LOS PRODUCTOS ALIMENTARIOS INTERMEDIOS PROCEDENTES DE LOS COPRODUCTOS DEL DÁTIL EN PRODUCTOS LÁCTEOS

3.5.1. Ingredientes

A continuación, se detallan las materias primas usadas en la elaboración de los distintos productos lácteos desarrollados para la presente tesis doctoral.

Para la elaboración de yogures, se utilizó leche semidesnatada de cabra adquirida en un supermercado local (Orihuela, Alicante, España) y el fermento comercial YO-MIX® 495 LYO 100 DCU (dosis de cultivo liofilizado necesaria para inocular o acidificar una determinada cantidad de leche) se obtuvo de Danisco (Copenhague, Dinamarca). Así como los productos alimentarios intermedios (PAI), en concreto pasta de dátil (TMP) y harina de dátil en estado tamar (TMH).

En la elaboración de queso fresco, se emplearon como materias primas leche de cabra de la granja de la Escuela Superior de Orihuela perteneciente a la Universidad Miguel Hernández (Orihuela, Alicante, España) y pasta de dátil en estado tamar (TMP). El cultivo iniciador CHOOZIT MA4001 se obtuvo de Danisco (Sassenage, Francia). El cloruro de calcio ($CaCl_2$) y el cuajo se obtuvieron de Laboratorios Arroyo (Santander, España).

Y por último para la elaboración de kéfir, se utilizaron como materias primas leche de cabra de la granja de la Escuela Superior de Orihuela perteneciente a la Universidad Miguel Hernández (Orihuela, Alicante, España), con la composición anteriormente proporcionada. Para su elaboración también se utilizó suero de la producción de los quesos frescos con pasta de dátil, con una composición de proteínas del 0,5 g/100 g; grasas del 0,3 g/100 g y carbohidratos del 4,1 g/100 g. También se usó la pasta de dátil en estado tamar (TMP). El cultivo iniciador (Kefir Yogotherm) se adquirió en Abaisa (Pontevedra, España) y contenía cepas de

Lactococcus lactis subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subps. *diacetylactis, Lactobacillus rhamnosus* y *Kluyveromyces marxianus*.

3.5.2. Yogures de leche de cabra fortificados con pasta y harina de dátil

3.5.2.1. Formulación y proceso de elaboración de los yogures

Se elaboraron cinco lotes de yogur con leche de cabra, con 16 yogures por cada lote. Uno de ellos se elaboró con una formulación tradicional que fue usado como control, (Tabla 5). En las otras cuatro formulaciones se adicionaron dos porcentajes (3% y 6%) de harina de dátil o de pasta de dátil. La elaboración se realizó por triplicado en tres días diferentes.

Tabla 5. Formulación de los yogures con adición de PAI de coproductos de dátil (harina y pasta) del cultivar Confitera.

Ingredientes	Control	Y3HD	Y6HD	Y3PD	Y6PD
Leche (mL)	1000	1000	1000	1000	1000
Fermento (mL)	0,32	0,32	0,32	0,32	0,32
Harina de dátil (g)	0	30	60	0	0
Pasta de dátil (g)	0	0	0	30	60

El porcentaje de los ingredientes viene referido sobre 1000 mL de leche. Y3HD: yogur con adición de un 3% de harina de dátil; Y6HD: yogur con adición de un 6% de harina de dátil; Y3PD: yogur con adición de un 3% de pasta de dátil; Y6PD: yogur con adición de un 6% de pasta de dátil.

Para elaborar los yogures, en primer lugar 8 litros de leche de cabra se dividieron en 5 partes iguales para establecer los lotes (1600 mL/lote). Una vez adicionada la pasta o harina de dátil en las cantidades mencionadas (Tabla 5), la mezcla se homogeneizó hasta alcanzar una temperatura de 42±2 ºC. Una vez alcanzada dicha temperatura, se le añadió el fermento de yogur en la concentración indicada por el fabricante (10-20 DCU/100 L) y se homogeneizó durante 2 minutos. Las mezclas se distribuyeron en recipientes estériles de 100 mL y se llevaron a una incubadora Memmert GmbH (Alemania) a una temperatura de 42±2 ºC hasta que se alcanzó un pH

final de entre 4,65 y 4,60. Tras este proceso de coagulación, los yogures se almacenaron entre 4-6 ºC por un periodo de 21 días. El proceso de elaboración se muestra en la figura 12.

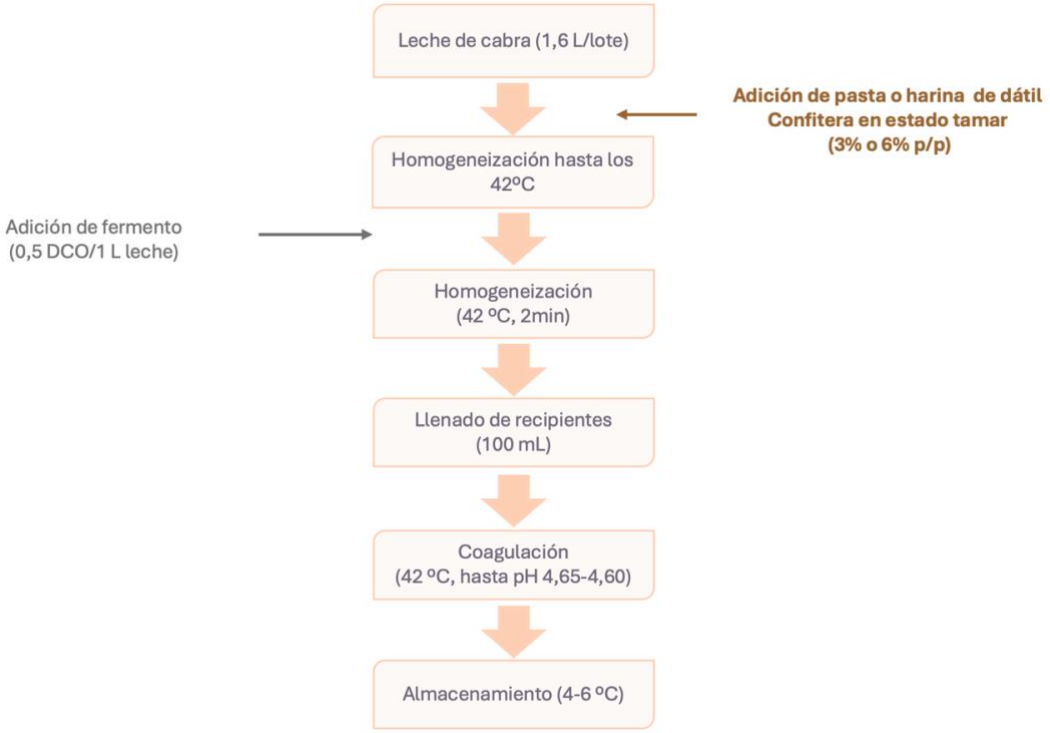


Figura 12. Diagrama de flujo de la elaboración de los yogures con adición de harina y pasta de dátil.

3.5.2.2. Caracterización de los yogures

3.5.2.2.1. Propiedades fisicoquímicas de los yogures

3.5.2.2.1.1. pH

La determinación de pH se realizó con la metodología y el equipo descrito con anterioridad en el apartado 3.1.3.1. La determinación se realizó en tres muestras de cada uno de los lotes de yogur.

3.5.2.2.1.2. Acidez titulable

La acidez titulable fue determinada mediante una valoración ácido-base con NaOH 0,11 N como disolución valorante y con fenolftaleína como indicador. Las determinaciones se realizaron por triplicado para cada uno de los lotes y los resultados vienen expresados en ºDornic (ºD).

3.5.2.2.1.3. Sinéresis

La sinéresis de las muestras se determinó siguiendo el método descrito por Jrad et al. (2022). Se usaron 50 mL de yogur (V_0) que se dejaron en reposo durante 6 h a 4ºC. Posteriormente se midió el volumen desuerado (V_1). El porcentaje de sinéresis se determinó mediante la ecuación 6 (Ec. 6):

$$\%\ sinéresis = \left(\frac{V_1}{V_0}\right) x100 \qquad \text{(Ec. 6)}$$

3.5.2.2.1.4. Parámetros de color

La determinación de los parámetros de color para los distintos lotes de yogures se llevó a cabo de igual forma que en lo descrito anteriormente en el apartado 3.1.3.2. Con la misma metodología y el mismo espectrofotocolorímetro. También se calculó el índice de blancura (IB), siguiendo la expresión proporcionada por Akgün et al. (2020) (Ec. 7). Se realizaron 18 medidas por lote de yogures a una temperatura aproximada de 25 ºC.

$$IB = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \qquad \text{(Ec. 7)}$$

3.5.2.2.1.5. Parámetros de textura

La textura de los yogures se midió durante los 21 días de almacenamiento en refrigeración siguiendo el método descrito por Silva y O'Mahony (2018) con ligeras modificaciones. Como se indicó con anterioridad, los yogures se guardaron a 4ºC en recipientes de polipropileno estériles de 100 mL de capacidad cuyas dimensiones concretas fueron de 55 mm de diámetro y 70 mm de altura. La textura se midió en intervalos de 7 días en un texturómetro TA-XT2i (Stable Micro System Ltd, Godalming, Surrey, Reino Unido), equipado con una célula de carga de 5 kg, utilizando una sonda cilíndrica de 10 mm de diámetro. Las condiciones de la sonda fueron: velocidad de 1,0 mm/s, alcanzando una profundidad de 30 mm trabajando a una temperatura ambiente de aproximadamente 25 ºC. Los parámetros de textura que se midieron fueron: firmeza (N), consistencia (N*s), cohesividad (N) y viscosidad (N*s). Dichos parámetros se obtuvieron de la fuerza positiva máxima de compresión, el área positiva de la curva (resistencia interna de las uniones dentro del producto), la fuerza negativa máxima de la curva (fuerza necesaria para retirar la sonda de la muestra) y el área negativa de la curva, correspondiéndose respectivamente con cada uno de los parámetros analizados. Se realizaron tres medidas por lote y por día analizado.

3.5.2.2.2. Composición química de los yogures

La composición química de los yogures se analizó por triplicado al día siguiente de su elaboración (día 0). El contenido de grasa, proteína y sólidos totales se midió con un MilkoScan FT120 (FOSS, Dinamarca) calibrado para nata. Mientras que la humedad y las cenizas se determinaron siguiendo los métodos de la AOAC (2010) detallados con anterioridad en los apartados 3.1.5.1. y 3.1.5.4.

3.5.2.2.2.1. Contenido de ácidos orgánicos y azúcares

La determinación del contenido de ácidos orgánicos y azúcares se realizó según lo descrito en el apartado 3.1.5.6. con modificaciones. Se pesaron 2,000±0,005 g de muestra y se le adicionaron 50 mL de agua ultrapura durante 24 h. El resto de las condiciones y del procedimiento fue el descrito en el apartado mencionado (3.1.5.6.). Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los lotes analizados de yogur y se realizaron en dos tiempos, en el momento inicial (día 0) y en el momento final (día 21).

3.5.2.2.2.2. Contenido en minerales

Se analizó el efecto de la adición de pasta y harina de dátil en la composición mineral del yogur mediante la metodología descrita en el apartado 3.1.5.7., incluida la liofilización previa de las muestras. Los resultados se obtuvieron tras analizar un triplicado de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de yogur.

3.5.2.2.3. Microbiología de los yogures

Se evaluó el efecto de la incorporación de los PAI de dátil en el cultivo iniciador del yogur durante 21 días de almacenamiento refrigerado a intervalos de 7 días, midiendo el número de células viables de *Lactobacillus spp*. y *Streptococcus spp*. Para estimar la población de *Lactobacillus spp*. y *Streptococcus spp.*, se llevó a cabo el mismo procedimiento detallado en el apartado 3.3.1.6. con pequeñas modificaciones. Se sembraron manualmente 0,1 ml, por duplicado, en agar MRS para *Lactobacillus spp.* y en agar M17 para *Streptococcus spp*. Después, las placas de Petri se incubaron

durante 48 h a 37ºC en el caso de *Streptococcus spp.* y a las mismas condiciones de temperatura y tiempo, pero en una cámara anaeróbica con Anaerocult A (Merck, Darmstadt, Alemania) para *Lactobacillus spp*. Con la finalidad de evaluar las condiciones higiénicas adecuadas durante la elaboración del yogur, también se determinaron los recuentos de mohos, levaduras y enterobacterias utilizando las mismas diluciones descritas anteriormente. Se sembró 1 ml de estas diluciones, por duplicado, en placas Petrifilm (3 M, Madrid, España) para mohos y levaduras y para enterobacterias. Las placas Petrifilm se incubaron durante 24 h a 37 ºC para las enterobacterias, y durante 120 h a 25 ºC para los mohos y las levaduras. Las placas que presentaron entre 30 y 300 unidades formadoras de colonias (UFC) se contaron manualmente y los resultados se expresaron como log UFC/g de yogur.

3.5.2.2.4. Análisis sensorial de los yogures

Para evaluar la aceptabilidad sensorial de los diferentes lotes de yogur, se reclutó a veintiséis consumidores (65 % mujeres y 35 % hombres, con edades comprendidas entre los 18 y los 65 años) en la Escuela Politécnica Superior de Orihuela perteneciente a la Universidad Miguel Hernández (UMH). Antes de comenzar los análisis, se informó a cada panelista sobre las características específicas del producto que se iba a valorar y degustar y todos los participantes firmaron un consentimiento informado por escrito. Todo el análisis sensorial se llevó a cabo con los lotes de yogures recién preparados (día 0) en el laboratorio sensorial estandarizado de la UMH, que cumple los requisitos de las normas internacionales (ASTM, 1986). Durante la evaluación, los panelistas se sentaron en cabinas privadas bajo luz fluorescente TL 5 (Philips-Ibérica, Madrid, España), con una intensidad de aproximadamente 350 lx. Las muestras de yogur (aproximadamente 10 g) se codificaron con un número aleatorio de 3 dígitos y se sirvieron a ciegas en un vaso de plástico transparente en un orden completamente aleatorio. El análisis sensorial de las distintas muestras de yogur se realizó con una escala hedónica de 9 puntos (1:

disgusta extremadamente a 9: me gusta extremadamente) para evaluar los atributos de olor, sabor, color, dulzor, acidez, firmeza, granulosidad y una pregunta final sobre aceptabilidad general.

3.5.2.2.5. Análisis estadístico

El proceso de elaboración de los yogures con adición de PAI de dátil variedad Confitera, se realizó por triplicado, cinco lotes independientes elaborados en tres días diferentes. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media más menos la desviación estándar de los datos obtenidos. Se comprobó la normalidad y la varianza constante de los datos antes de aplicar el análisis ANOVA. Se calculó el ANOVA de un factor (utilizando un nivel de confianza del 95 %) para determinar cualquier diferencia significativa entre los lotes de yogures. Se aplicó un ANOVA de dos factores (con un nivel de confianza del 95 %) para evaluar la estabilidad de los yogures a lo largo del tiempo (utilizando el tratamiento y el tiempo de almacenamiento como efectos fijos). Cuando se observó una diferencia significativa ($p < 0,05$), se realizó la prueba de Tukey para determinar las diferencias entre las diferentes formulaciones de yogur y los tiempos de almacenamiento. El análisis estadístico de los datos se realizó utilizando SPSS (IBM SPSS Statistics versión 26).

3.5.3. Queso fresco de leche de cabra fortificado con pasta de dátil

3.5.3.1. Formulación y proceso de elaboración de los quesos frescos

Para la elaboración de los quesos frescos, inicialmente se pasteurizó la leche de cabra (60 L) durante 15 s a una temperatura de 72 ºC. Posteriormente se separaron en tres lotes de 20 L cada uno. Uno de ellos se elaboró con una formulación tradicional el cual se estableció como formulación control, otro lote se realizó con una adición del

4% de pasta de dátil (DP4) y al último se le adicionó un 8% de pasta de dátil (DP8) (Tabla 6).

Tabla 6. Formulación de los quesos frescos con adición de pasta de dátil.

Ingredientes	Control	DP4	DP8
Leche de cabra (mL)	1000	1000	1000
Cultivo iniciador (mL)	1	1	1
Cuajo (mL)	1	1	1
$CaCl_2$ (mL)	0,25	0,25	0,25
Sal (g)	7	7	7
Pasta de dátil (g)	0	40	80

El porcentaje de los ingredientes viene referido sobre 1000 mL de leche. DP4: queso fresco con adición de un 4% de pasta de dátil; DP8: queso fresco con adición de un 8% de pasta de dátil.

Para elaborar los quesos frescos, en primer lugar, la leche tras pasteurizarla se enfrió hasta una temperatura de 30 ºC, se les adicionó 0,05 unidades de cultivo iniciador Danisco (DCU/L). A continuación, se incorporó la pasta de dátil en las cantidades correspondientes para cada lote, seguido de la adición del cuajo microbiano 1:15000, el $CaCl_2$ y la sal (Tabla 6). Una vez añadidos todos los ingredientes, se dejaron las cubas coagular durante 45 min, tras este tiempo se cortó y se removió durante 10 min. Por último, se moldearon los quesos y se mantuvieron en una cámara de maduración Oscar Zarzosa (España) en condiciones de refrigeración (4-8 ºC) y humedad del 85% hasta su posterior análisis. El suero del queso se recogió y guardó en botellas de 1,5 L de tereftalato de polietileno (PET) y se mantuvo en condiciones de congelación (-18 ºC) para su posterior uso. La figura 13 muestra el proceso de elaboración de los quesos.

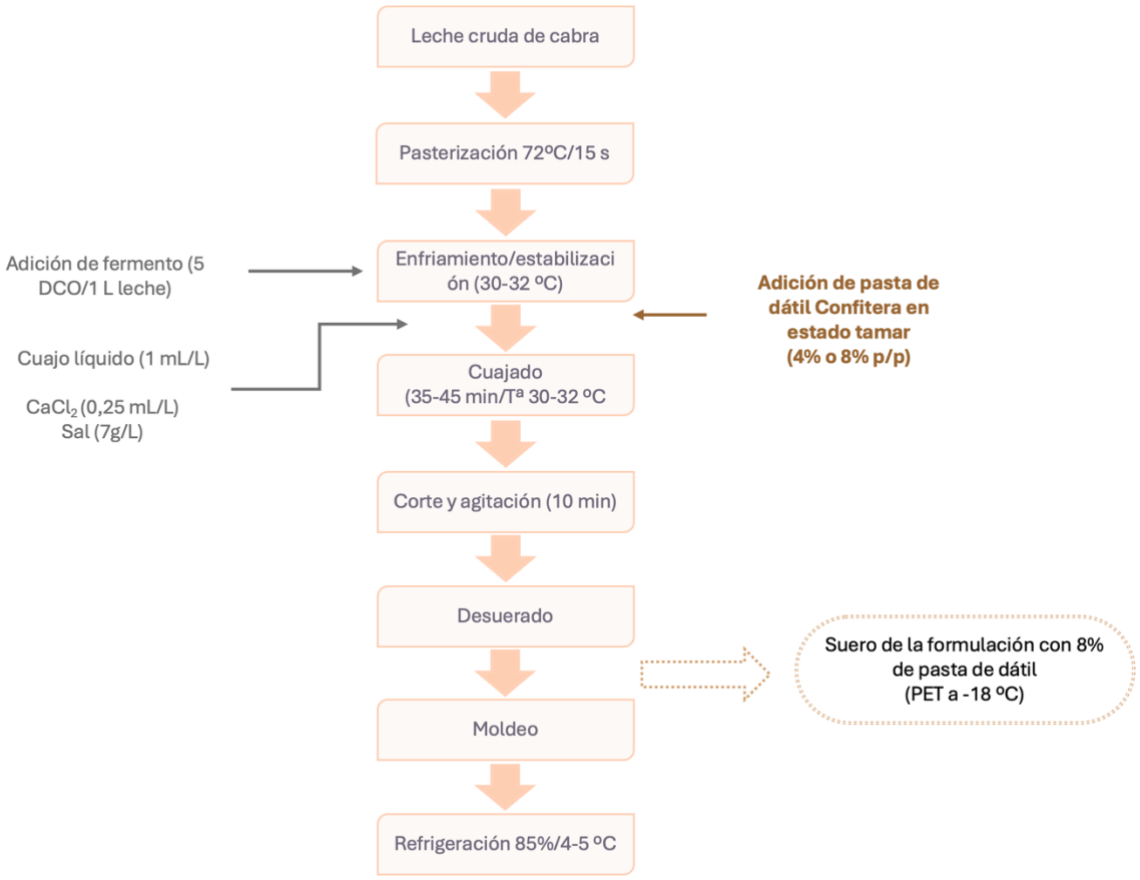


Figura 13. Diagrama de flujo de la elaboración de los quesos frescos con adición de PAI de coproductos de dátil (pasta) del cultivar Confitera.

3.5.3.2. Caracterización de los quesos frescos

Tras la elaboración, los tres lotes de queso fresco fueron analizados. La primera determinación que se le realizó fue el cálculo del rendimiento de los mismos. El rendimiento se calculó mediante la ecuación 8 (EC. 8).

$$\%\ rendimiento = \frac{Kg\ de\ queso}{Kg\ de\ leche}\ x\ 100 \qquad \text{(Ec. 8)}$$

3.5.3.2.1. Propiedades fisicoquímicas de los quesos frescos

3.5.3.2.1.1. pH

El pH se determinó en un pH-metro Sension +ph31 (Hach, España) equipado con un electrodo de punción. Las medidas se realizaron por triplicado para cada uno de los lotes analizados.

3.5.3.2.1.2. Actividad de agua

La actividad de agua de las muestras se midió con un higrómetro Novasina TH-200 (Novasina, Lanchen, Suiza) a 25 ºC. La determinación se realizó por triplicado para cada uno de los lotes de queso fresco.

3.5.3.2.1.3. Parámetros de color

Los parámetros de color para los tres lotes de queso fresco se determinaron bajo las mismas condiciones, con la misma metodología y con la misma equipación que lo descrito con anterioridad en el apartado 3.1.3.2. Aparte de los valores de L*, a* y b*, se determinaron el croma (C*), el tono (h*), las diferencias de color (ΔE*) y el índice de blancura (IB) mediante las ecuaciones 1, 2, 3 y 7 respectivamente. Se realizaron 18 medidas para cada lote de quesos frescos.

3.5.3.2.1.4. Parámetros de textura

Para la determinación de la textura de los quesos frescos, se sometió a las muestras a un análisis del perfil de textura (TPA). Para dicho TPA se usó un texturómetro TA-XT2i (Stable Micro System Ltd., Godalming, Surrey, UK) equipado con una célula de carga de 5 kg y una sonda cilíndrica de aluminio P/100. Las muestras de queso,

cortadas en cubos de 1,5 cm, fueron sometidas a dos ciclos de compresión del 25% y a una velocidad de 1,3 mm/s, con un tiempo de 5 s de recuperación entre ambas compresiones. Los parámetros texturales que se midieron fueron dureza (N), adhesividad (N*s), elasticidad, cohesividad y resiliencia. Se realizaron cinco medidas por lote analizado.

3.5.3.2.2. Composición química de los quesos frescos

3.5.3.2.2.1. Cenizas

El contenido de cenizas se determinó por el método correspondiente de la AOAC (2010), detallado en el apartado 3.1.5.1. Las determinaciones se realizaron por triplicado para cada uno de los lotes de queso fresco y los resultados se expresaron como g de cenizas/100 g de muestra.

3.5.3.2.2.2. Proteína

La cantidad de proteína de los quesos frescos se determinó mediante el método Kjeldahl (981.10) de la AOAC (2010), con un factor de conversión de 6,38 correspondiente a leche y productos lácteos.

3.5.3.2.2.3. Humedad

La humedad de las muestras de los tres lotes de quesos frescos se determinó por el método correspondiente de la AOAC (2010) descrito con anterioridad en el apartado 3.1.5.5. La determinación se realizó por triplicado y los resultados se expresaron como g de agua/100 g de muestra.

3.5.3.2.2.4. Grasa total

El contenido de grasa total de las muestras de quesos frescos se determinó mediante el método Gerber con un butirómetro. Para llevarlo a cabo, se usaron 10 mL de ácido sulfúrico y 1 mL de alcohol amílico añadidos a 5 g de muestra. La determinación se realizó por triplicado y los resultados se expresaron como g de grasa/100 g de muestra.

3.5.3.2.2.5. Contenido de ácidos orgánicos y azúcares

La determinación del contenido de ácidos orgánicos y azúcares se realizó según lo descrito con anterioridad en el apartado 3.1.5.6. con modificaciones. Se pesaron 1,000±0,005 g de queso fresco y se le adicionaron 25 mL de agua ultrapura durante 24 h. El resto de las condiciones y del procedimiento fue el descrito en el apartado anteriormente mencionado (3.1.5.6.). Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los lotes analizados de queso fresco.

3.5.3.2.2.6. Contenido en minerales

Se analizó el efecto de la adición de pasta en la composición mineral del queso fresco mediante la metodología descrita en el apartado 3.1.5.7., incluida la liofilización previa de las muestras. Los resultados se obtuvieron tras analizar un triplicado de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de queso fresco.

3.5.3.2.2.7. Ácidos grasos

El perfil de ácidos grasos de los lotes de queso fresco se determinó mediante una extracción y metilación lipídica. El contenido graso de las muestras se extrajo por medio del método Folch et al. (1957). Tras la extracción, la fase lipídica se metiló según el método de Pellegrini et al. (2018a). Se analizaron estos ésteres metílicos de ácidos grasos (AGME) utilizando un cromatógrafo de gases HP 6890 (Tecknokroma, Barcelona, España), equipado con detector de ionización de llama (FID) y una columna capilar Suprewax-280 (0,25 mm de diámetro interno, 30 m de longitud y película de 0,25 µm; Tecknokroma, Barcelona, España). La temperatura del inyector fue de 250 °C mientras que la del detector se estableció en 270 °C. El programa de temperatura consistió en iniciar a 60 °C y mantenerlo durante 1 min., luego aumentarla a una tasa de 10 °C/min hasta 170 °C. Después de mantener esta temperatura durante 2 min, se elevó el horno a razón de 3 °C/min hasta alcanzar 230 °C, donde se mantuvo durante 10 min. Finalmente, se incrementó la temperatura hasta 260 °C a una velocidad de 2 °C/min y se mantuvo durante 1 min. La presión interna en la columna fue de 11 psi y se inyectaron 0,2 µL en modo *split*. El gas portador utilizado fue helio. Se emplearon estándares de AGME para comparar los tiempos de retención. A partir de los cromatogramas, se calcularon el total de ácidos grasos saturados (AGS), monoinsaturados (AGMI) y poliinsaturados (AGPI). Las determinaciones se realizaron por triplicado para cada uno de los lotes de queso fresco y los resultados de los ácidos grasos se expresaron en g de ácido graso/100 g de grasa total.

3.5.3.2.3. Análisis microbiológico de los quesos frescos

Se evaluó el efecto de la incorporación de la pasta de dátil en los lotes de quesos frescos sobre el desarrollo de las bacterias lácticas (células viables de *Lactobacillus spp*. y *Streptococcus spp)* así como la determinación de mohos, levaduras y enterobacterias para evaluar las condiciones higiénicas durante la

elaboración de los quesos frescos. Para estimar la población de *Lactobacillus spp.*, *Streptococcus spp.*, mohos y levaduras y enterobacterias, se llevó a cabo el mismo procedimiento detallado en el apartado 3.5.2.2.3. Las placas que presentaron entre 30 y 300 UFC se contaron manualmente y los resultados se expresaron como log UFC/g de queso fresco.

3.5.3.2.4. Análisis sensorial de los quesos frescos

Para evaluar la aceptabilidad sensorial de los diferentes quesos frescos con adición de pasta de dátil, se reclutó a cincuenta consumidores (60 % mujeres y 40 % hombres, con edades comprendidas entre los 18 y los 65 años) pertenecientes al personal y estudiantes de la Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández (UMH). Antes de comenzar los análisis, se informó a cada miembro sobre las características específicas del producto que se iba a valorar y degustar y todos los participantes firmaron un consentimiento informado por escrito. Todo el análisis sensorial se llevó a cabo con los lotes de quesos frescos recién preparados, en el laboratorio sensorial estandarizado de la UMH, que cumple los requisitos de las normas internacionales (ASTM, 1986). Las muestras de queso fresco cortadas en porciones de 2 x 2 cm de la parte interna del queso, se codificaron con un número aleatorio de 3 dígitos y se sirvieron a ciegas en platos de plástico en un orden completamente aleatorio. El análisis sensorial de las distintas muestras de yogur se realizó con una escala hedónica de 9 puntos (1: disgusta extremadamente a 9: me gusta extremadamente) para evaluar 9 atributos seleccionados basados en lo más apreciado por los consumidores de queso y que pudiera variar con la adición de la pasta de dátil. Los atributos evaluados fueron: olor, sabor, color, dulzor, sabor salado, firmeza, fracturabilidad, granulosidad y una pregunta final sobre aceptabilidad general.

3.5.3.2.5. Microscopia con láser de barrido Confocal de los quesos frescos

La microestructura de las muestras de queso fresco de cabra se analizaron utilizando un microscopio de láser de barrido Confocal Leica SP5 (TCS-SPE, Leica Microsystems, Heidelberg, Alemania) siguiendo el método descrito por Muñoz-Tebar et al. (2022) con ligeras modificaciones. Las muestras se cortaron en secciones de 95 µm de grosor con un micrótomo HM400 (Microm, Walldorf, Alemania) y se sumergieron en una combinación de Fast Green FCF (68724 Supelco, Merck KGaA, Darmstadt, Alemania) y Rojo Nilo (72485 Sigma-Aldrich, Merck KGaA Darmstadt, Alemania) durante 10 min. De ambos colorantes se prepararon disoluciones madre (1 mg/ml en agua desionizada para Fast Green FCF y en Lectinas FITC y dimetilsulfóxido (DMSO) para Rojo Nilo. A continuación, estas soluciones madre se diluyeron con agua desionizada y DMSO para alcanzar una concentración final de 0,1 mg/ml justo antes de la tinción. El Rojo Nilo se excitó a 488 nm, mientras que el Fast Green FCF se excitó a 633 nm. Los filtros de emisión se ajustaron a 520-590 nm para el Rojo Nilo y las lectinas FITC, y a 660-750 nm para el Fast Green FCF.

3.5.3.2.6. Análisis estadístico

El proceso de elaboración de los quesos frescos con adición de pasta de dátil en una proporción de 4% y 8%, se realizó por triplicado, tres lotes independientes elaborados en tres días diferentes para las tres muestras. Se aplicó un ANOVA de un factor con un nivel de confianza del 95 % para identificar cualquier diferencia significativa entre los diferentes lotes. Cuando se encontraron diferencias significativas ($p < 0,05$), se realizó la prueba de Tukey para identificar diferencias específicas entre las formulaciones de queso fresco. Todos los análisis se realizaron utilizando 5 muestras independientes ($n = 5$) y todos los ensayos se llevaron a cabo por triplicado, excepto el análisis de textura (10 repeticiones). El análisis de los datos se realizó utilizando SPSS (IBM SPSS Statistics versión 26).

3.5.4. Kéfir de leche de cabra fortificado con pasta de dátil y suero de queso fresco con pasta de dátil

3.5.4.1. Formulación y proceso de elaboración del kéfir

Se realizaron cinco lotes de kéfir elaborado con leche de cabra. Uno de ellos se elaboró con una formulación tradicional, que fue usado como kéfir control (Tabla 7). En otros dos de los lotes a la formulación tradicional se adicionaron dos porcentajes (3% y 6%) de pasta de dátil variedad Confitera en estado de maduración tamar (DP3 y DP4). Los últimos dos lotes se elaboraron reemplazando un 25% y un 50% de leche de cabra por suero de queso fresco con una adición del 8% de pasta de dátil elaborado en el apartado 3.5.3.1. (WH25 y WH50 respectivamente). La elaboración se realizó por triplicado en tres días diferentes.

Tabla 7. Formulación de kéfir con adición de pasta de dátil y de suero de queso fresco (con pasta de dátil) como sustituto de leche.

Ingredientes	Control	DP3	DP6	WH25	WH50
Leche (mL)	1000	1000	1000	750	500
Suero (mL)	0	0	0	250	500
Cultivo iniciador (g)	1,0	1,0	1,0	1,0	1,0
Pasta de dátil (g)	0	30	60	0	0

El porcentaje de los ingredientes viene referido sobre 1000 mL de leche de cabra. DP3: kéfir con adición de un 3% de pasta de dátil; DP6: kéfir con adición de un 6% de pasta de dátil; WH25: kéfir con un reemplazo del 25% de leche de cabra por suero de queso con adición de un 8% de pasta de dátil; WH50: kéfir con un reemplazo del 50% de leche de cabra por suero de queso con adición de un 8% de pasta de dátil.

Para elaborar los kéfires, en primer lugar, se pasteurizaron 5 L de leche de cabra durante 30 min a 60 ºC en una Thermomix TM6 Vorwerk (Wuppertal, Alemania) y posteriormente se enfrió a 25 ºC. La cantidad de leche se dividió en 5 partes iguales para establecer los lotes (1000 mL/lote). Para los dos últimos lotes únicamente se adicionaron 750 mL/lote (WH25) y 500 mL/lote (WH50) de leche de cabra respectivamente. La pasta de dátil se mezcló con la leche o con el suero de queso y, a continuación, se añadió el cultivo iniciador de kéfir en la dosis establecida por la

empresa (1 g/L de leche). Posteriormente, el kéfir se vertió en recipientes de 100 ml y se incubó durante 20-22 h a 25 ºC. Por último, las muestras se refrigeraron durante aproximadamente 24 h hasta su posterior análisis. El proceso de elaboración se muestra en la figura 14.

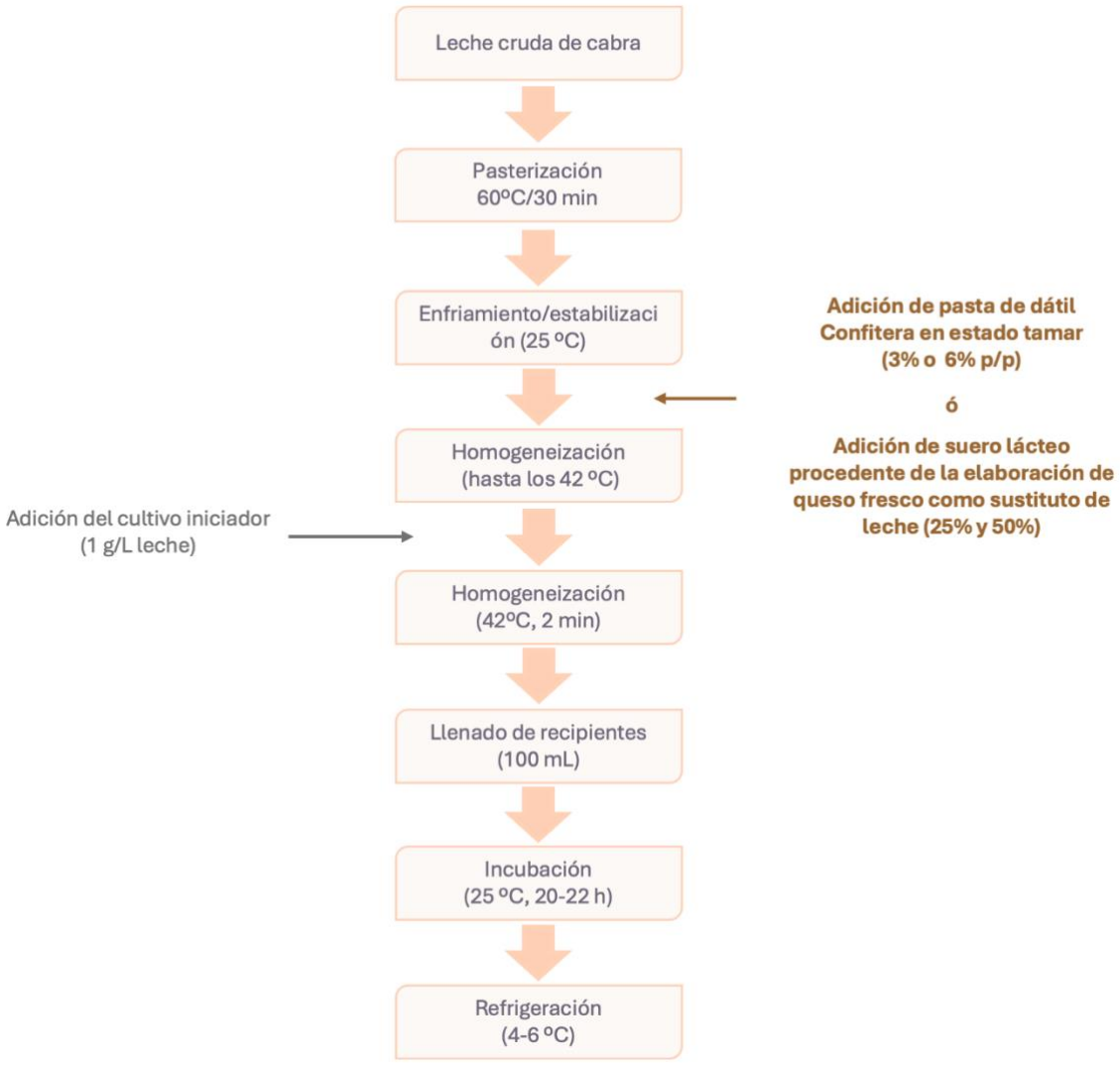


Figura 14. Diagrama de flujo de la elaboración de kéfir con adición de PAI de coproductos de dátil (pasta) del cultivar Confitera y uso de suero de queso como sustituto de leche.

3.5.4.2. Caracterización del kéfir

3.5.4.2.1. Propiedades fisicoquímicas del kéfir

3.5.4.2.1.1. pH

El pH de los cinco lotes de kéfir se determinó con la misma metodología, pH-metro descrito en el apartado anterior 3.5.3.2.1.1. Las determinaciones se realizaron por triplicado para cada uno de los lotes de kéfir.

3.5.4.2.1.2. Acidez titulable

La acidez titulable de los cinco lotes de kéfir fue determinada mediante una valoración ácido-base, como se describe en el apartado 3.5.2.2.1.2. Las determinaciones se realizaron por triplicado para cada uno de los lotes y los resultados vienen expresados en ºDornic (ºD).

3.5.4.2.1.3. Parámetros de color

Los parámetros de color para los tres lotes de queso fresco se determinaron bajo las mismas condiciones, con la misma metodología y con la misma equipación que la descrita con anterioridad en el apartado 3.1.3.2. A parte de los valores de L*, a* y b*, se determinaron el croma (C*), el tono (h*) y el índice de blancura (IB) mediante las ecuaciones 1, 2, 3 y 7 respectivamente. Se realizaron 18 medidas para cada lote de quesos frescos.

3.5.4.2.1.4. Viscosidad

La viscosidad de los cinco lotes de kéfir se determinó por triplicado con el viscosímetro rotacional J.P. Selecta ST-2020-L (Barcelona, España) con el husillo S2 a 40 rpm según la metodología propuesta por Paredes et al. (2022). Los resultados se expresaron en mPa.s.

3.5.4.2.2. Composición química del kéfir

La humedad (925.45) y las cenizas (AOAC 923.03) se analizaron siguiendo los métodos AOAC (2010) (detallados en los apartados 3.1.5.1. y 3.1.5.4.). Por otro lado, la grasa, las proteínas y los sólidos totales se determinaron con un MilkoScan FT120 (FOSS, Hilleroed, Dinamarca) (detallado en el apartado 3.5.2.2.2.). Todas las medidas para cada lote de kéfir se realizaron por triplicado.

3.5.4.2.2.1. Contenido de ácidos orgánicos y azúcares

La determinación del contenido de ácidos orgánicos y azúcares se realizó según lo descrito con anterioridad en el apartado 3.1.5.6. con modificaciones. Se pesaron 2,000±0,005 g de kéfir y se le adicionaron 50 mL de agua ultrapura durante 24 h. El resto de las condiciones y del procedimiento fueron descritas en el apartado 3.1.5.6. Los resultados para los ácidos orgánicos se expresaron como mg de ácido orgánico/100 g de muestra, mientras que para los azúcares fue expresado como g de azúcar/100 g de muestra. Las determinaciones se realizaron por triplicado para cada uno de los lotes analizados de kéfir.

3.5.4.2.2.2. Contenido en minerales

Se analizó el efecto de la adición de pasta de dátil en la composición mineral del kéfir mediante la metodología descrita en el apartado 3.1.5.7., incluida la liofilización previa de las muestras. Los resultados se obtuvieron tras analizar un triplicado de cada uno de los lotes. El contenido final de cada mineral se expresó en mg/100 g de peso seco de kéfir.

3.5.4.2.3. Análisis microbiológico del kéfir

Se evaluó el efecto de la incorporación de la pasta de dátil y del suero de queso en los cultivos iniciadores de los kéfires y en la calidad higiénica. Para ello se determinó el número de células viables de *Lactobacillus spp*. y *Streptococcus spp* así como de mohos, levaduras y enterobacterias. Para estimar la población de *Lactobacillus spp*., *Streptococcus spp.*, mohos y levaduras y enterobacterias, se llevó a cabo el mismo procedimiento detallado en el apartado 3.4.2.2.3. Las placas que presentaron entre 30 y 300 UFC se contaron manualmente y los resultados se expresaron como log UFC/g de kéfir.

3.5.4.2.4. Análisis sensorial del kéfir

Para evaluar la aceptación del kéfir con adición de pasta de dátil se llevó a cabo un análisis sensorial en la Escuela Politécnica de Orihuela de la Universidad Miguel Hernández (UMH) con cincuenta consumidores (55 % mujeres, 45 % hombres, con edades comprendidas entre los 20 y los 65 años). Antes de comenzar los análisis, se informó a todos los participantes sobre las características distintivas del producto que iban a degustar y la naturaleza del análisis en sí. Asimismo, los participantes dieron su consentimiento informado por escrito. Las muestras de kéfir se sirvieron en vasos pequeños de plástico transparentes, cada uno etiquetado con un código de tres dígitos

diferentes y se sirvieron en orden aleatorio. Los consumidores evaluaron los siguientes siete atributos: color, olor, sabor, dulzor, acidez, viscosidad y una última pregunta sobre aceptabilidad general. Se utilizó una escala hedónica de nueve puntos (1: disgusta extremadamente a 9: me gusta extremadamente) (Ramírez-Rivera et al., 2018).

3.5.4.2.5. Análisis estadístico

El proceso de elaboración de los lotes de kéfir se realizó por triplicado, cinco lotes independientes elaborados en tres días diferentes. Cada lote fue analizado por triplicado. Los resultados obtenidos se expresaron como la media más menos la desviación estándar. Se realizó un ANOVA de un factor (con un nivel de confianza del 95 %) sobre los resultados obtenidos para determinar si existían diferencias significativas entre los diferentes lotes. Cuando se observaron diferencias significativas, se realizó una prueba de Tukey para identificar los lotes diferentes. Los análisis estadísticos se realizaron utilizando el programa estadístico SPSS (IBM SPSS Statistics versión 26).

3.6. DIGESTIBILIDAD *IN VITRO* DE LA PASTA DE DÁTIL Y EL QUESO FRESCO ELABORADO CON LECHE DE CABRA Y PASTA DE DÁTIL

Se analizó la estabilidad y bioaccesibilidad de los compuestos polifenólicos tanto de la pasta de dátil (TMP) como del queso fresco con pasta de dátil, tras someter a las muestras a una digestión *in vitro*.

3.6.1. Estabilidad y bioaccesibilidad de compuestos polifenólicos

La extracción de polifenoles se llevó a cabo en cuatro matrices diferentes: pasta de dátil en estado tamar (TMP) y los tres lotes de queso fresco elaborados con leche de cabra (Control, DP4 y DP8). Se llevaron a cabo dos metodologías diferentes, una para

obtener los polifenoles libres (referidos como polifenoles libres solubles) y compuestos unidos insolubles.

3.6.1.2. Extracción de los compuestos polifenólicos libres

Para la extracción de (poli)fenoles libres, se siguió el procedimiento descrito por Pellegrini et al. (2018b) con algunas modificaciones. En primer lugar, se pesaron 5 g de cada muestra y se mezclaron con 50 ml de disolución de metanol y agua (80:20 v/v). A continuación, para facilitar la homogeneización, las muestras se mezclaron durante 1 minuto con un T-25 Digital Ultraturrax (IKA Works, España) y luego se centrifugaron durante 10 minutos a 7000 g a 4 °C. El sobrenadante se depositó en un matraz de 250 mL y se repitió el proceso, pero sustituyendo el extractante por 50 ml de una disolución de acetona y agua (70:30; v/v). Los sobrenadantes se mezclaron y se llevaron a sequedad en un rotavapor (Büchi Rotavapor R-200, Büchi Ibérica, Barcelona, España). Tras esta etapa, se redisolvió en 5 mL de agua ultrapura. Para minimizar cualquier posible interferencia por el contenido de azúcares de las muestras durante el análisis por cromatografía, se utilizó un cartucho C-18 Sep-Pak (Thermo Scientific™ Cartridge HyperSep™ C18, Fisher Scientific, Madrid, España), activado previamente con 3 mL de metanol grado HPLC, 3 mL de agua ultrapura y 3 mL de ácido clorhídrico 0,01 N. Posteriormente el cartucho se lavó con 5 mL de agua ultrapura, la elución de la muestra se llevó a cabo con 0,5 mL de metanol grado HPLC acidificado al 0,1 g/L de ácido fórmico. Los extractos resultantes se conservaron cuidadosamente a −18 ºC hasta el análisis por HPLC.

3.6.1.3. Extracción de los compuestos polifenólicos unidos

Para la extracción de los compuestos polifenólicos unidos, se utilizó el sedimento obtenido en la extracción de polifenoles libres (3.6.1.2.), siguiendo la metodología descrita por Lucas-González et al. (2018) con algunas modificaciones. Al

sedimento se le añadió 40 ml de NaOH (4 M) y se agitó durante 4 horas. A continuación, se ajustó el pH de la muestra a 2,0 con HCl (6 M) y se centrifugó durante 20 min a 10000 g y 4 ºC. El sobrenadante se transfirió a embudos de decantación y se le añadieron 30 ml de acetato de etilo, se agitó durante 2 minutos y se dejó durante 24 horas para separar las fases. El sobrenadante se lavó con 20 ml de acetato de etilo y se evaporó en un rotavapor. Finalmente, las muestras se recuperaron en 10 ml de agua, se pasaron por una columna C18 y se resuspendieron en 0,5 ml de metanol acidificado (ácido fórmico al 1 %).

3.6.2. Detección e identificación de los compuestos polifenólicos

La detección de los compuestos polifenólicos fue llevada a cabo usando cromatografía líquida de alta resolución (HPLC) siguiendo el procedimiento descrito por Lucas-González et al. (2018). Las muestras se analizaron usando una columna C18 Mediterranean Sea18 (25 x 0,4 cm, con un tamaño de partícula de 5 µm) (Teknokroma, Barcelona, España), en un equipo cromatográfico Hewlett-Packard HPLC serie 1200. Se aplicó un volumen de inyección de 20 µL de muestra y un gradiente de elución de 1 mL/min de dos fases móviles: Fase A: ácido fórmico al 0,1% (agua:ácido fórmico; 99:1), y la fase móvil B: acetonitrilo de grado HPLC. Se trabajó con tres longitudes de onda: 280, 320 y 360 nm. Previamente se inyectaron 19 patrones siguiendo las mismas condiciones: Los estándares individuales de (poli)fenoles y la mezcla de catequinas de té verde (G-016), compuesta por (+)-catequina, (–)-catequina-3-galato, (–)-epicatequina, (–)-epicatequina-3-galato, (–)-epigalocatequina-3-galato, (–)-galocatequina y (–)-galocatequina-3-galato (Merck, Darmstadt, Alemania). La mezcla de seis monoglucósidos (pelargonidina-3-glucósido, cianidina-3-glucósido, peonidina-3-glucósido, delfinidina-3-glucósido, petunidina-3-glucósido y malvidina-3-glucósido), junto con malvidina y malvidina-3,5-diglucósido (Biolink Group–Polyphenols AS, Sandnes, Noruega). Asimismo, se utilizaron los siguientes compuestos fenólicos y flavonoides: ácido 4-hidroxibenzoico, apigenina, apigenina-7-

O-glucósido, ácido cafeico, catequina, catequina-3-galato, ácido cinámico, ácido cripto-clorogénico, cianidina-3-glucopiranósido, delfinidina-3-O-β-glucopiranósido, diosmetina-7-O-rutinósido (diosmina), diosmetina-7-O-neohesperidósido (neodiosmina), ácido elágico, epicatequina, epicatequina-3-galato, epigalocatequina-3-galato, erioticitol-7-O-rutinósido (eriocitrina), ácido ferúlico, ácido gálico, galocatequina-3-galato, galocatequina galato, hesperetina-7-rhamnoglucósido (hesperidina), isoramnetina-3-O-glucósido, kaempferol, L-triptófano, luteolina, malvidina, malvidina-3-O-β-glucopiranósido, malvidina-3,5-di-O-β-glucopiranósido, malvidina-3-O-glucopiranósido, miricetina, naringenina-7-O-neohesperidósido (naringina), naringenina-7-O-rutinósido (narirutina), ácido p-cumárico, pelargonidina-3-O-β-glucopiranósido, peonidina-3-O-β-glucopiranósido, petunidina-3-O-β-glucopiranósido, isosakuranetina-7-O-neohesperidósido (poncirina), ácido protocatecuico, quercetina, quercetina-3-β-D-glucósido, quercetina-3-O-ramnósido (quercitrina), quercetina-3-O-rutinósido (rutina), ácido rosmarínico, ácido sinápico, ácido siríngico, ácido vainílico y vainillina (PanReac AppliChem, Barcelona, España). Los compuestos fueron identificados comparando el tiempo de retención y el espectro de absorbancia con los patrones. La cuantificación se realizó por regresión lineal. Los resultados se obtuvieron tras analizar un triplicado de dos muestras de cada uno de los lotes. Los resultados se expresaron como µg compuesto fenólico/g muestra.

3.6.3. Digestión *in vitro*

Para la digestión *in vitro* se siguió el procedimiento descrito en el protocolo INFOGEST 2.0 (Brodkorb et al. 2019). El procedimiento se inició empleando una proporción 1:1, equivalente a 5 g de muestra y 5 ml de solución salival simulada sin α-amilasa. Posteriormente, la mezcla se agitó con el fin de favorecer su homogeneización y reproducir el proceso de masticación. Las muestras se incubaron en un baño orbital a 37 °C y 70 rpm durante 2 min. Seguidamente, se incorporaron 10 ml de solución gástrica simulada (pepsina, 2000 U/mL), se ajustó el pH a 3,0 y se mantuvieron en

incubación durante 2 horas en el mismo sistema. Posteriormente, el pH se elevó a 7,0 y se añadieron 20 ml de solución intestinal simulada (pancreatina: 100 U/mL de tripsina, 100 U/mL de amilasa, 8 U/mL de lipasa; sales biliares, 10 mM), incubándose nuevamente durante 2 horas. Finalmente, las muestras fueron centrifugadas a 7000 rpm durante 10 minutos. El sobrenadante obtenido se destinó a la identificación de polifenoles libres, mientras que el sedimento se sometió a un procedimiento de extracción de polifenoles no extraíbles.

3.6.4. Estabilidad y bioaccesibilidad tras la digestión *in vitro*

La estabilidad de los polifenoles tras la digestión gastrointestinal *in vitro* se evaluó mediante el índice de recuperación total (Ec. 9), el índice de bioaccesibilidad y el índice de disponibilidad en el colon. Para el cálculo de la bioaccesibilidad, se siguió la fórmula recomendada por Brodkorb et al. (2019). Tanto la bioaccesibilidad como el índice de disponibilidad en el colon se calcularon utilizando las ecuaciones 10 y 11.

$$Índice\ de\ recuperación\ (\%) = \frac{PC_{DF}}{PC_{TM}} x\ 100 \qquad \text{(Ec. 9)}$$

Donde,

PC_{DF}: contenido de fenoles totales en la fracción digerida después de cada fase de digestión

PC_{TM}: contenido de fenoles totales cuantificados en la matriz de prueba.

$$Índice\ de\ bioaccesibilidad\ (\%) = \frac{CSF_i}{FT_u} x\ 100 \qquad \text{(Ec. 10)}$$

Donde,

CSF: fracción soluble en quimo

FT: contenido fenólico total en la muestra digerida total

$$Índice\ de\ disponibilidad\ en\ el\ colon\ (\%) = \frac{BF_i}{BP_c} x100 \quad \text{(Ec. 11)}$$

Donde,

BF: fracción bioaccesible (cantidad de compuesto disponible tras la digestión gastrointestinal y que puede llegar al colon).

BP: Porción basal (la cantidad inicial o de referencia del compuesto presente antes de la digestión).

3.6.5. Análisis estadístico

Todos los análisis se realizaron por triplicado. Se realizó un ANOVA unidireccional (con un nivel de confianza del 95 %) sobre los resultados obtenidos para determinar si existían diferencias significativas entre las muestras y se realizó una prueba de Tukey para comprobar las diferencias significativas obtenidas ($p < 0,05$). Para el análisis estadístico se utilizó el software SPSS (IBM SPSS Statistics versión 26).

CAPÍTULO 4.
RESULTADOS Y DISCUSIÓN



4. RESULTADOS Y DISCUSIÓN

En el presente capítulo se muestra un resumen de los resultados más destacados de esta Tesis Doctoral. El conjunto completo y extenso de resultados y discusión se muestran en los artículos recogidos en el capítulo 7.

4.1. CARACTERIZACIÓN DE LOS DÁTILES PROCEDENTES DE LOS DOS CULTIVARES COMERCIALES MÁS RELEVANTES EN EL PALMERAL DE ELCHE (MEDJOUL Y CONFITERA)

La tabla 8 muestra los resultados del estudio de las características morfológicas de los dátiles de los dos cultivares estudiados (Medjoul y Confitera).

Tabla 8. Características morfológicas de los dátiles de los cultivares Medjoul y Confitera cultivados en España.

	Longitud (cm)	Ancho (cm)	Peso total (g)	Peso pulpa (g)	Peso piel (g)	Peso semilla (g)
Medjoul	3,88±0,36^{b}	2,14±0,25^{a}	11,64±3,85^{a}	9,08±3,16^{b}	1,15±0,37^{a}	1,23±0,45^{a}
Confitera	4,58±0,22^{a}	2,14±0,17^{a}	13,12±1,61^{a}	10,97±1,48^{a}	1,09±0,20^{a}	0,97±0,17^{b}

Las diferentes letras $^{a-b}$ entre columnas muestran diferencias significativas ($p < 0,05$) entre los cultivares estudiados. Los datos se representan como media y DS.

En dicha tabla (tabla 8) se puede observar que los dátiles de ambos cultivares no presentaron diferencias significativas ($p > 0,05$) en el ancho (2,14 – 2,14 cm), ni en el peso total del fruto (11,64 – 13,12 g), ni en el peso de la piel del fruto (1,15 – 1,09 g). En cambio, en la longitud del fruto se obtuvo una variabilidad entre los cultivares con valores de 3,88 cm para Medjoul y 4,58 cm para Confitera. A pesar de no existir diferencias en el peso total de ambos cultivares, se observaron diferencias significativas ($p < 0,05$) en cuanto al peso de la pulpa de los mismos, siendo los dátiles de Confitera los que obtuvieron valores mayores con respecto a Medjoul (10,97g, 9,08g respectivamente), lo que corresponde con un rendimiento del 84% para los dátiles Confitera y un 78% para Medjoul. En cuanto al peso de la semilla, fueron los dátiles Medjoul los que obtuvieron valores mayores a Confitera (1,23 – 0,97 g). Por un lado, es cierto que no existen referencias bibliográficas para los dátiles del cultivar ilicitano Confitera, pero en cambio, las características morfológicas de los dátiles Medjoul son bastante semejantes con los resultados

aportados por otros estudios (Martín-Sánchez et al., 2010; Muralidhara et al., 2016; Salomón-Torres et al., 2019).

Teniendo en cuenta que el cultivar Confitera es bastante desconocido, se buscó realizar una caracterización lo más completa posible, que permita no solo comparar ambos cultivares, sino aportar información útil tanto para su comercialización como para su industrialización.

Los datos de pH y propiedades de color de los dátiles de ambos cultivares se presentan en la tabla 9.

Tabla 9. Propiedades fisicoquímicas de los dátiles de los cultivares Medjoul y Confitera procedentes en España.

	pH	L*	a*	b*	C*	h
Medjoul	5,74±0,02^{b}	32,50±1,11^{a}	4,39±0,65^{b}	4,96±1,04^{b}	6,63±1,20^{b}	48,22±2,04^{a}
Confitera	5,94±0,04^{a}	32,36±0,30^{a}	5,13±0,35^{a}	5,85±0,33^{a}	7,78±0,36^{a}	48,72±2,32^{a}

Las diferentes letras $^{a-b}$ entre columnas muestran diferencias significativas ($p < 0,05$) entre los cultivares estudiados. Los datos se representan como media y DS.

El color es un parámetro muy importante, ya que se utiliza de referencia de calidad y valor comercial de las frutas. En numerosos estudios se detalla que las variaciones de color en el dátil están totalmente relacionadas con el cultivar y el proceso de maduración (Almeida et al., 2015; Ghnimi et al., 2017; Al-Qarni y Bazzi, 2020), donde la clorofila se degrada a lo largo de las diversas etapas, proporcionándole al dátil la tonalidad amarillo-marrón que lo caracteriza (Al-Qarni y Bazzi, 2020; Al-Okbi, 2022). Como puede observarse en la tabla 9, se aprecian diferencias significativas en las coordenadas a* (tonalidad rojo-verde), b* (tonalidad azul-amarillo) y C* (el cual hace referencia a la saturación o intensidad del color), siendo Confitera el cultivar con los valores más altos. A pesar de no existir diferencias significativas entre los dátiles de ambos cultivares con respecto al tono, el cual representa un tono rojo anaranjado (IRANOR, 1991), Confitera destaca por tener un color más intenso y vibrante que Medjoul. En cuanto al pH, los dátiles Confitera mostraron valores más elevados (5,94) con respecto a los Medjoul (5,74), aun así, ambos se encuentran en el rango establecido para los dátiles en el

estado de maduración tamar, el cual abarca de 5,2 a 6,3 (Al-Hooti et al., 1997; Borchani et al., 2010). Esto está estrechamente relacionado con el contenido en ácidos orgánicos que contienen ambos cultivares, teniendo valores más altos de estos ácidos los dátiles Confitera (Tabla 10) donde destacan principalmente el ácido tartárico (Medjoul: 6,93 mg/g; Confitera: 15,42 mg/g), ascórbico (Medjoul: 4,06 mg/g; Confitera:7,42 mg/g) y succínico (Medjoul: 12,19 mg/g; Confitera: 15,75 mg/g).

En la tabla 10, se presenta la composición proximal, contenido de minerales, azúcares y ácidos orgánicos de los dátiles de ambos cultivares. Los dátiles Medjoul mostraron valores de humedad más altos que los dátiles Confitera, lo que puede atribuirse tanto al cultivar como a las condiciones de crecimiento y cultivo (lugar, clima, el agua, etc.) (Al-Hooti et al., 1997; Al-Farsi y Lee, 2008). No se apreciaron diferencias significativas ni en el contenido de fibra dietética total ni de cenizas ($p > 0,05$). En cambio, los dátiles Confitera mostraron valores más elevados de proteínas (2,58 g/100 g) y de azúcares totales (47,59 g/100 g) con respecto a los Medjoul (1,98 g/100 g; 43,17 g/100 g respectivamente), mientras que para el contenido en grasa fue al revés: los dátiles Confitera mostraron valores inferiores (0,18 g/100 g) con respecto a los Medjoul (0,37 g/100 g). En referencia a los azúcares, destacaron glucosa y fructosa, al igual que en numerosos estudios (Borchani et al., 2010; Martín-Sánchez et al., 2014; Salomón-Torres et al., 2019; Tassoult et al., 2021), y no se apreciaron diferencias significativas entre cultivares ($p > 0,05$). De acuerdo con (Al-Hooti et al., 1997; Rastegar et al., 2012), el contenido de minerales en los dátiles disminuye a lo largo de las diferentes etapas de maduración, pero aún así, incluso en la última etapa (tamar), el dátil es una fuente importante de minerales esenciales. A excepción del calcio y el cobre, que no mostraron diferencias significativas ($p > 0,05$), el resto de los minerales estudiados obtuvieron cantidades mayores en los dátiles Confitera con respecto a Medjoul, siendo los más destacados potasio, magnesio y sodio. Además, cabe destacar la proporción de sodio:potasio, que de acuerdo con (Turck et al., 2019), se encuentra

en armonía con las recomendaciones dietéticas actuales relacionadas con la reducción del riesgo de enfermedades cardiovasculares (EFSA, 2019).

Tabla 10. Composición proximal y mineral y perfil de azúcares y ácidos orgánicos de los dátiles procedentes de los cultivares Medjoul y Confitera cultivados en España.

	Medjoul	**Confitera**
Composición proximal (g/100g)		
Humedad	32,38±0,52^{a}	25,65±0,65^{b}
Proteínas	1,98±0,05^{b}	2,58±0,18^{a}
Grasa	0,37±0,05^{a}	0,18±0,04^{b}
FDT	20,05±0,81^{a}	21,94±0,94^{a}
Cenizas	2,05±0,07^{a}	2,06±0,03^{a}
Azúcares	43,17±0,67^{b}	47,59±0,76^{a}
Composición mineral (mg/100g)		
K	639,39±47,67^{b}	837,33±68,82^{a}
Mg	89,82±5,37^{b}	114,65±7,41^{a}
Ca	100,62±5,50^{a}	98,65±1,72^{a}
Na	30,68±0,61^{b}	43,15±0,85^{a}
Fe	1,20±0,01^{b}	1,70±0,30^{a}
Zn	0,83±0,08^{b}	0,99±0,09^{a}
Cu	0,65±0,07^{a}	0,62±0,03^{a}
Mn	0,39±0,02^{b}	0,50±0,01^{a}
Azúcares (mg/g)		
Glucosa	279,57±1,73^{a}	274,52±3,57^{a}
Fructosa	260,73±1,61^{a}	262,78±1,2^{a}
Ácidos orgánicos (mg/g)		
Oxálico	1,15±0,02^{a}	1,22±0,06^{a}
Tartárico	6,93±0,6^{b}	15,42±0,66^{a}
Málico	7,93±0,33^{a}	8,21±0,46^{a}
Ascórbico	4,06±0,18^{b}	7,42±0,10^{a}
Succínico	12,19±0,24^{b}	15,75±0,11^{a}

Las diferentes letras $^{a\text{-}b}$ entre columnas muestran diferencias significativas ($p < 0,05$) entre los cultivares estudiados. Los datos se representan como media y DS.

Las propiedades tecnofuncionales de los alimentos tienen un papel fundamental en el desarrollo de alimentos ya que determinan el comportamiento de este durante la elaboración, almacenamiento, consumo y vida útil. En este caso,

las propiedades estudiadas fueron la capacidad de retención de agua (CRA), la capacidad de retención de aceite (CRO) y la capacidad de hinchamiento (SWC).

Las propiedades de hidratación de los alimentos, CRA y SWC, son importantes tanto por su papel fisiológico como por su impacto en sus características tecnofuncionales. Por un lado, la CRA hace referencia a la cantidad de agua que puede retener una matriz sin liberarla (jugosidad), mientras, la SWC está relacionada con el volumen que puede aumentar un alimento al absorber el agua (viscosidad, gelificación o sensación de saciedad) (Acquah et al., 2021; Seidy et al., 2021). Estos parámetros ofrecen información clave sobre la habilidad de la matriz alimentaria para hidratarse, lo que permite comprender su comportamiento durante el tránsito intestinal y en los procesos de transformación de los alimentos (Dhingra et al., 2012; López-Marcos et al., 2015). Además, existen estudios que muestran que matrices con valores elevados de CRA y SWC pueden incorporarse como ingredientes funcionales en el diseño de alimentos con valor añadido, debido a su efecto beneficioso en la digestión, el cual está relacionado con la absorción de agua en el intestino y un incremento del volumen fecal, lo que favorece el tránsito intestinal (Viuda-Martos et al., 2010; Sahni y Shere, 2017). A pesar de estos beneficios, hay que tener en cuenta que una alta afinidad con el agua puede afectar negativamente tanto a la textura del alimento como a su vida útil (Sharoba et al., 2013; Sahni y Shere, 2017). Tanto los dátiles Medjoul como los Confitera mostraron valores similares ($p > 0,05$) de ambas propiedades (CRA y SWC). En cambio, la CRO está relacionada con la cantidad de aceite que puede retener la matriz, relacionada con la estructura química y física de los polisacáridos, sus propiedades superficiales y su porosidad (Sánchez-Zapata et al., 2009; López-Marcos et al., 2015). No se encontraron diferencias significativas entre los dátiles de los dos cultivares estudiados ($p > 0,05$), con valores similares a otros vegetales como piña o manzana (Chia y Chong, 2015).

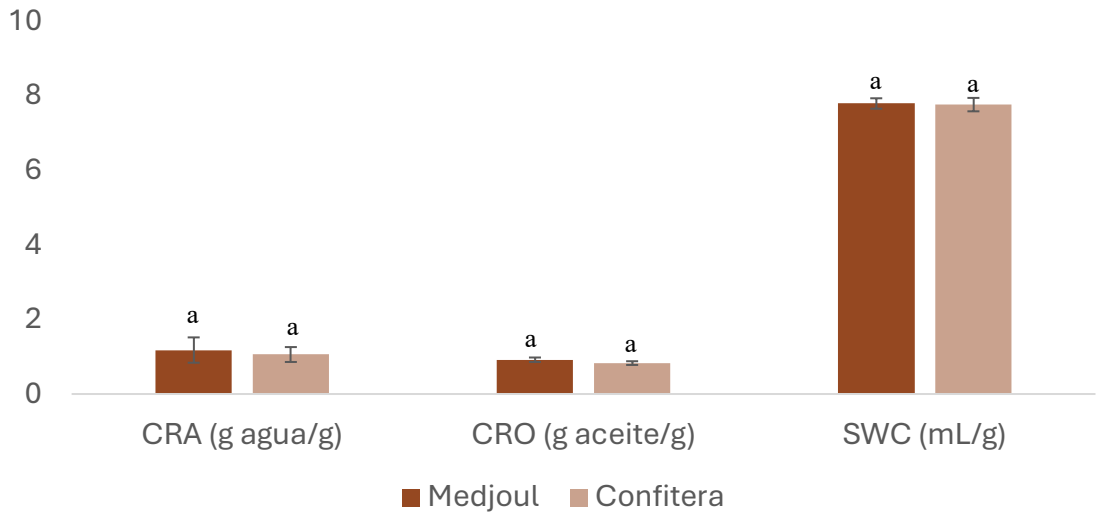


Figura 15. Propiedades tecnofuncionales de los dátiles procedentes de los cultivares Medjoul y Confitera cultivados en España.
Misma letra minúscula encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad.

De acuerdo con numerosos estudios, los dátiles destacan por ser una fuente de antioxidantes, de los cuales destacan los compuestos polifenólicos como los flavonoides y los fenoles. Cabe destacar que la presencia de estos compuestos en los dátiles se ve influido por el cultivar, el estado de maduración, la localización y las condiciones ambientales (Hadrami et al., 2011; Eid et al., 2013; Hussain et al., 2020; Fernández-López et al., 2022). En la tabla 11 se encuentran los compuestos polifenólicos estudiados, de los cuales siete de ellos son flavonoides y un ácido fenólico. Los dátiles Confitera presentaron concentraciones más elevadas de epicatequina, catequina, eriocitrina, hesperidina y rutina, pero menores niveles de luteolina-7-O-glucósido e isoquercitrina en comparación con los dátiles de Medjoul. Mientras, en el ácido cafeico no se detectaron diferencias estadísticamente significativas entre ambos cultivares ($p > 0,05$; Medjoul: 3,37 μg/g y Confitera: 3,32 μg/g), a pesar de esto, este flavonoide se ha identificado en varios estudios como uno de los compuestos polifenólicos predominantes en numerosas cultivares de dátiles independientemente de su origen (Mansouri et al., 2005; Hamad et al., 2015; El-Arem et al., 2017).

La actividad antioxidante de los dátiles de Confitera y Medjoul se estudió a través de cuatro métodos diferentes ya que dicha actividad se puede desarrollar por

distintos mecanismos (tabla 11). Los cuatro métodos estudiados fueron DPPH (capacidad de las muestras para atrapar el radical DPPH o donar Hidrógenos), FRAP (mide la capacidad de las moléculas para reducir el complejo férrico basado en la transferencia de electrones), FIC (determina la capacidad quelante de las muestras) y ABTS (capacidad de las muestras para atrapar el radical catiónico ABTS). Los dátiles Confitera mostraron mayor actividad antioxidante por los métodos DPPH, FRAP y FIC y menor actividad por el método ABTS que los dátiles Medjoul ($p < 0,05$). Numerosos estudios reportaron que la actividad antioxidante de los dátiles está estrechamente relacionada con su contenido total en compuestos polifenólicos, principalmente flavonoides (Allaith, 2008; Hamad et al., 2015; Abdul-Hamid et al., 2020; Echegaray et al., 2020; Tassoult et al., 2021), hecho que también se observó en este estudio, donde los dátiles Confitera mostraron una mayor actividad antioxidante total debido a un mayor contenido en flavonoides que los dátiles Medjoul.

Tabla 11. Compuestos bioactivos presentes en los dátiles de los cultivares Medjoul y Confitera cultivados en España y sus propiedades antioxidantes.

	Medjoul	**Confitera**
Compuestos polifenólicos (µg/g)		
(-) Epicatequina	ND	1241,90±1,13
Luteolina-7-O-glucósido	26,61±0,74^{a}	8,21±0,47^{b}
(+) Catequina	ND	1150,89±1,86
Ácido cafeico	3,37±0,09^{a}	3,32±0,06^{a}
Eriocitrina	8,76±0,23^{b}	10,85±0,12^{a}
Herperidina	7,47±0,32^{b}	16,76±0,41^{a}
Rutina	11,00±0,48^{b}	12,97±0,63^{a}
Isoquercitrina	19,03±0,74^{a}	10,43±0,36^{b}
Propiedades antioxidantes		
FRAP (mg TE/g pulpa)	0,64±0,05^{b}	0,78±0,06^{a}
DPPH (µg TE/g pulpa)	26,46±2,11^{b}	32,33±3,13^{a}
FIC (µg EDTA/g pulpa)	3,75±0,39^{b}	4,63±0,87^{a}
ABTS (mg TE/g pulpa)	1,14±0,28^{a}	0,91±0,14^{b}

Las diferentes letras $^{a-b}$ entre columnas muestran diferencias significativas ($p < 0,05$) entre los cultivares estudiados. Los datos se representan como media y DS. ND: no detectado.

Según los resultados obtenidos y teniendo en cuenta que ambos cultivares estudiados se cultivaron bajos las mismas condiciones, se puede concluir que los dátiles del cultivar autóctono Confitera mostraron una mayor calidad global en sus frutos en comparación con Medjoul. Los dátiles Confitera mostraron una longitud y un rendimiento de pulpa mayor que los dátiles Medjoul. Desde el punto de vista nutricional, se apreció que el contenido en minerales y en compuestos polifenólicos era superior en los dátiles Confitera. En base a todos estos resultados y al hecho de que el cv. Confitera es endémico en el Palmeral de Elche, se decidió a partir de aquí ya centrar todo el estudio en los coproductos procedentes de la comercialización del dátil fresco cv. Confitera.

4.2. CARACTERIZACIÓN DE LOS PRODUCTOS DE ALTO VALOR AÑADIDO (PRODUCTOS ALIMENTARIOS INTERMEDIOS) OBTENIDOS TRAS EL PROCESAMIENTO DE LOS COPRODUCTOS DEL DATIL CONFITERA EN DIFERENTES ESTADOS DE MADURACIÓN

Los coproductos de los dátiles Confitera se procesaron aplicando únicamente tratamientos físicos (molienda, remojo, prensado y secado) sin necesidad de aplicar ningún otro tipo de tratamiento, lográndose de esta forma la obtención de tres productos alimentarios intermedios (PAI) de alto valor añadido: pasta, harina y agua de dátil, cada uno de ellos procedente de los coproductos de dátiles procesados en diferente estado de maduración (khalal, rutab y tamar).

4.2.1. Caracterización de la pasta de dátil

A continuación, en la tabla 12 se muestran los resultados obtenidos tras el estudio de la composición proximal de la pasta de dátiles Confitera procedente del procesamiento de los coproductos de dátiles en tres estados de maduración diferentes (khalal, rutab y tamar).

Las pastas obtenidas mostraron diferencias significativas en los resultados de humedad, FDT y azúcares. Se observó cómo, tanto los valores de humedad como de fibra dietética iban disminuyendo durante la maduración, en cambio, el

contenido en azúcares fue aumentando ($p < 0,05$). Esto es debido a que, durante las etapas de maduración, los dátiles sufren una serie de cambios. En la etapa khalal, el contenido en agua de los dátiles ronda el 60-70%, disminuyendo al 30-45% cuando pasa a la etapa rutab y quedando en torno al 10-25% de contenido de agua en la última etapa de maduración, tamar (Mohammed et al., 2021; El-Beltagi et al., 2023). En cambio, el aumento del contenido en azúcares es debido no solo a la disminución de agua, sino también a la degradación enzimática durante la maduración (Echegaray et al., 2021; Fernández-López et al., 2022).

En cuanto al contenido en minerales de las diferentes pastas de dátiles, no se apreció una tendencia clara con respecto a la fase de maduración ya que en algunos minerales (Mg, Zn y Mn) el contenido no se vio afectado ($p > 0,05$), mientras que en otros como el potasio, el hierro, el calcio y el cobre aumentaron y el sodio disminuyó ($p < 0,05$).

Las pastas de dátiles se caracterizan por su elevado contenido en azúcares, siendo la glucosa y la fructosa —ambos azúcares reductores— los predominantes (tabla 12). La cantidad y el tipo de azúcares presentes dependen tanto del cultivar como de la etapa de maduración (Al-Qarni y Bazzi, 2020). El contenido de azúcares reductores está estrechamente vinculado a la textura y al color. Además, dichos azúcares contribuyen a una mayor suavidad del fruto y pueden originar cambios de color en las pastas cuando se someten a tratamientos térmicos, debido a su participación como sustratos en reacciones de Maillard (Ghnimi et al., 2018).

En este estudio, el contenido de azúcares reductores en las pastas aumentó significativamente con el grado de maduración del fruto, alcanzando su valor máximo en TMP, tanto en glucosa como en fructosa, coincidiendo con los resultados obtenidos en otros estudios (Hamad et al., 2015).

Por otro lado, los ácidos orgánicos son muy importantes debido a su efecto en la reducción del pH, su función como conservantes naturales y su efecto en el sabor de la pasta de dátiles (acidez). Teniendo en cuenta que el cultivar, las condiciones

de crecimiento y la etapa de maduración afecta al contenido en ácidos orgánicos (Sánchez-Zapata et al., 2011; Martín-Sánchez et al., 2014; Famiani et al., 2015) se observó como el contenido de estos disminuyó durante la maduración, siendo los ácidos tartárico, málico y succínico los predominantes tanto en KHP como en TMP.

Tabla 12. Composición proximal y perfil de minerales, azúcares y ácidos orgánicos de las pastas de dátil procedentes de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	KHP	RTP	TMP
Composición proximal (g/100g)			
Humedad	54,67±0,42^{a}	51,09±0,31^{b}	48,25±0,33^{c}
Proteínas	1,13±0,08^{a}	1,11±0,01^{a}	1,16±0,01^{a}
Grasa	0,25±0,13^{a}	0,17±0,01^{a}	0,39±0,33^{a}
FDT	21,99±0,23^{a}	18,87±0,29^{b}	18,61±0,23^{b}
Cenizas	0,62±0,42^{a}	0,64±0,09^{a}	0,48±0,22^{a}
Azúcares	21,34±0,26^{c}	28,12±0,14^{b}	31,11±0,22^{a}
Composición mineral (mg/100g)			
K	613,76±11,06^{c}	655,21±14,55^{b}	658,28±4,72^{a}
Mg	130,96±13,46^{a}	130,79±1,6^{a}	127,55±2,20^{a}
Ca	121,25±4,16^{c}	143,87±4,51^{a}	135,64±2,24^{b}
Na	4,43±0,22^{a}	1,43±0,09^{c}	2,12±0,17^{b}
Fe	1,21±0,06^{b}	1,13±0,01^{c}	1,32±0,04^{a}
Zn	0,59±0,06^{a}	0,57±0,01^{a}	0,54±0,04^{a}
Cu	0,73±0,04^{b}	0,69±0,00^{b}	0,79±0,02^{a}
Mn	0,66±0,05^{a}	03,61±0,02^{a}	0,65±0,03^{a}
Azúcares (mg/g)			
Glucosa	112,41±5,17^{c}	168,79±0,48^{b}	177,81±4,60^{a}
Fructosa	105,00±2,95^{c}	113,84±5,53^{b}	137,72±8,52^{a}
Ácidos orgánicos (mg/g)			
Oxálico	0,01±0,00^{a}	0,02±0,00^{a}	0,01±0,00^{a}
Tartárico	5,03±0,14^{a}	ND	0,62±0,05^{b}
Málico	4,23±0,06^{a}	4,76±0,18^{a}	3,65±0,23^{b}
Cítrico	1,49±0,02^{a}	0,27±0,04^{b}	0,20±0,03^{b}
Succínico	15,20±0,06^{a}	15,72±0,50^{a}	10,53±0,63^{b}

$^{a\text{-}c}$Para el mismo compuesto, las diferentes letras en la misma fila indican diferencias significativas entre las muestras (etapas de maduración) ($p < 0,05$); ND: no detectado; KHP: pasta de dátiles en la etapa khalal; RTP: pasta de dátiles en la etapa rutab; TMP: pasta de dátiles en la etapa tamar.

En la tabla 13 se muestran las propiedades fisicoquímicas de las pastas de dátiles, en ella se puede observar cómo KHP mostró valores de pH menores ($p < 0,05$) que RTP y TMP, las cuales mostraron valores similares ($p > 0,05$). Esto está

estrechamente relacionado con el contenido en ácidos orgánicos, mostrados en la tabla anterior (tabla 12), donde una mayor presencia de estos proporciona mayor acidez, lo que produce una disminución del pH.

E l color es uno de los aspectos más afectados por el proceso de maduración de la fruta y, en el caso de los dátiles, estos cambios son más evidentes, ya que se utilizan para su clasificación en las diferentes etapas de maduración (khalal, rutab y tamar). En cuanto a la luminosidad (L*) de las pastas, esta disminuyó a medida que avanzaba la maduración de los dátiles de procedencia, obteniendo los valores L* más altos en las pastas de dátiles en la fase khalal (KHP) y los más bajos en la fase tamar (TMP), siendo esta última más oscura que la RTP y la KHP ($p < 0,05$). Varios autores han descrito una relación entre la humedad y la luminosidad de los alimentos, donde a mayor cantidad de agua, mayor es la reflexión de la luz y, por lo tanto, mayor es la luminosidad (Vera-Zambrano et al., 2019). En el resto de coordenadas (a*, b*, C* y h), se siguió la misma tendencia, donde KHP y RTP mostraron valores superiores a TMP ($p < 0,05$). Además, los cambios que tuvieron lugar en a* y b* se deben principalmente a la degradación de la clorofila, al contenido en carotenos y antocianinas y a reacciones no enzimáticas como la reacción de Maillard (Al-Qarni y Bazzi, 2020).

Tabla 13. Propiedades fisicoquímicas de las pastas de dátil procedentes de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	KHP	RTP	TMP
pH	6,46±0,21^{b}	7,22±0,01^{a}	7,16±0,01^{a}
L*	40,32±0,11^{a}	34,85±0,50^{b}	30,29±0,73^{c}
a*	4,35±0,26^{a}	4,48±0,20^{a}	2,51±0,09^{b}
b*	10,73±0,61^{a}	9,99±0,74^{a}	4,91±0,18^{b}
C*	11,58±0,63^{a}	10,95±0,75^{a}	5,51±0,18^{b}
h	67,90±1,13^{a}	65,79±0,78^{a}	62,87±1,11^{b}

$^{a-c}$Para el mismo parámetro, las diferentes letras en la misma fila indican diferencias significativas entre las muestras (etapas de maduración) ($p < 0,05$). L* luminosidad, a* coordenada rojo/verde, b* coordenada amarillo/azul, C* croma, H* tono. Pasta de dátiles KHP en etapa khalal, pasta de dátiles RTP en etapa rutab, pasta de dátiles TMP en etapa tamar.

4.2.2. Caracterización del agua de dátil

En la siguiente tabla (tabla 14) se muestra el perfil de minerales de las aguas de dátil obtenidas tras los lavados de la pasta. Al igual que ocurre en la pasta, los minerales mayoritarios fueron calcio, potasio y magnesio. Además, en común con otros estudios, estos minerales también han sido descritos como los principales en varios zumos de frutas como el limón, la naranja, la uva, el mango, la piña, la sandía, etc (Ichado y Ayeni, 2020; Oladipo et al., 2022). Cabe destacar que el potasio fue el único mineral que tuvo valores superiores a 1 g/L en las aguas del primer lavado de los tres estados de maduración estudiados. En general, como se muestra en la tabla 14, el contenido de minerales disminuyó significativamente con el número de lavados, lo que respalda el hecho de que la mayor extracción de minerales se produce durante el primer lavado. Finalmente, se podría decir que el agua de dátil es una buena fuente de minerales debido al perfil obtenido.

En la tabla 15 se muestra el perfil de azúcares y ácidos orgánicos de las diferentes aguas procedentes del procesamiento de los coproductos de dátiles analizados. Se puede observar que el contenido en azúcares extraídos fue mayor en el primer lavado y fue disminuyendo progresivamente con el resto de lavados. Los contenidos más altos de azúcar corresponden a las aguas de los coproductos de los dátiles más maduros (TMA). Además, de los dos azúcares extraídos, glucosa y fructosa, esta última fue la que mostró resultados mayores ($p < 0,05$). En comparación con otros estudios, también se detectó la glucosa y la fructosa como los azúcares mayoritarios en las aguas de cítricos (Viuda-Martos et al., 2010). En los ácidos orgánicos detectados (oxálico, cítrico, tartárico, málico y succínico) se observa que los valores obtenidos disminuyeron progresivamente con los lavados, al igual que los azúcares. El ácido tartárico fue el único que no fue detectado en las aguas procedentes de los coproductos de dátiles en el estado de maduración tamar. Además, en comparación con otros estudios, las aguas de cítricos y membrillo también fueron capaces de extraer gran parte de los ácidos orgánicos que se encontraban en las pastas de dichas frutas (Viuda-Martos et al. 2010; Trigueros et al. 2011).

Tabla 14. Perfil de minerales (mg/100mL) del agua de dátil procedente de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
KHA1	152,98±2,12[a]	0,48±0,02[a]	0,75±0,07[a]	1010,02±14,14[a]	212,50±0,71[a]	0,76±0,02[a]	19,70±0,28[a]	0,70±0,14[a]
KHA2	90,75±0,21[b]	0,33±0,03[b]	0,85±0,08[a]	614,50±21,92[b]	110,50±3,64[b]	0,43±0,01[b]	14,55±0,21[b]	0,67±0,09[a]
KHA3	54,50±2,55[c]	0,17±0,01[c]	0,82±0,12[a]	238,00±12,73[c]	33,15±0,78[c]	0,18±0,01[c]	3,53±0,25[c]	0,25±0,06[b]
RTA1	178,54±3,54[a]	1,25±0,01[a]	1,44±0,14[a]	1425,04±7,07[a]	269,00±4,24[a]	0,55±0,01[a]	8,76±1,04[a]	0,84±0,04[a]
RTA2	110,50±6,36[b]	0,68±0,06[b]	0,86±0,18[b]	813,50±3,54[b]	137,50±6,36[b]	0,32±0,06[b]	4,66±0,16[b]	0,61±0,09[b]
RTA3	48,35±6,86[c]	0,27±0,01[c]	0,60±0,02[c]	297,00±2,83[c]	41,30±0,28[c]	0,14±0,01[c]	1,82±0,01[c]	0,28±0,10[c]
TMA1	155,00±3,78[a]	1,02±0,01[a]	1,12±0,03[a]	1300,06±113,14[a]	252,50±7,78[a]	0,58±0,01[a]	5,49±0,65[a]	0,74±0,09[a]
TMA2	94,65±3,04[b]	0,64±0,01[b]	0,91±0,01[b]	732,50±42,98[b]	130,50±4,85[b]	0,38±0,01[b]	4,17±0,47[b]	0,59±0,05[b]
TMA3	48,15±4,60[c]	0,34±0,01[c]	1,06±0,07[a]	321,00±14,14[c]	41,95±1,20[c]	0,21±0,01[c]	1,96±0,07[c]	0,40±0,11[c]

[a-c]Para la misma muestra (KHA, RTA o TMA) y mineral, las diferentes letras en las columnas indican diferencias significativas entre los lavados ($p < 0,05$). KHA1: agua de dátiles en la fase khalal tras el primer lavado; KHA2: agua de dátiles en la fase khalal tras el segundo lavado; KHA3: agua de dátiles en la fase khalal tras el tercer lavado; RTA1: agua de dátiles en la fase rutab tras el primer lavado; RTA2: agua de dátiles en la fase rutab tras el segundo lavado; RTA3: agua de dátiles en la fase rutab tras el tercer lavado; TMA1: agua de dátiles en la fase tamar del primer lavado; TMA2: agua de dátiles en la fase tamar tras el segundo lavado; TMA3: agua de dátiles en la fase tamar tras el tercer lavado, y ND: no detectado.

Tabla 15. Perfil de azúcares y ácidos orgánicos (mg/100mL) del agua de dátil procedente de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	Azúcares (mg/100 mL)		Ácidos orgánicos (mg/100mL)				
	Glucosa	**Fructosa**	**Ácido oxálico**	**Ácido cítrico**	**Ácido tartárico**	**Ácido málico**	**Ácido succínico**
KHA1	6918,60±14,21^{aY}	7468±100,34^{aX}	253,22±0,05^{aA}	4,59±0,05^{aD}	1,06±0,05^{E}	95,82±0,73^{aB}	39,82±0,44^{aC}
KHA2	5281,09±299,65^{bX}	5914,34±117,39^{bX}	193,83±1,44^{bA}	2,70±0,89^{bD}	ND	68,92±0,53^{bB}	24,52±0,56^{bC}
KHA3	5684,52±279,54^{bX}	3741,85±1,31^{cY}	114,89±2,02^{cA}	2,95±0,00^{bD}	ND	24,36±0,05^{cB}	15,47±0,06^{cC}
RTA1	9539,85±45,90^{aY}	10346,38±37,41^{aX}	285,52±2,43^{aA}	4,25±0,26^{aD}	3,19±0,73^{aD}	151,91±0,98^{aC}	41,50±0,96^{aB}
RTA2	9574,31±175,81^{aX}	6763,45±126,36^{bY}	178,01±3,93^{bA}	3,12±0,31^{bD}	2,54±0,09^{bD}	90,93±0,34^{bB}	27,41±0,62^{bC}
RTA3	6249,51±64,93^{bX}	3397,67±14,56^{cY}	81,90±0,08^{cA}	1,45±0,04^{cD}	1,83±0,12^{cD}	47,53±10,67^{cB}	11,78±0,75^{cC}
TMA1	9367,91±426,12^{aY}	10430±182,21^{aX}	334,86±3,17^{aA}	5,58±0,17^{aD}	ND	160,94±2,87^{aB}	48,46±1,71^{aC}
TMA2	9508,09±234,10^{aX}	8844,52±113,30^{bY}	219,03±0,68^{bA}	4,69±0,05^{bD}	ND	105,73±0,86^{bB}	27,41±0,29^{bC}
TMA3	6400,42±170,17^{bY}	7173,54±94,24^{cX}	86,88±0,76^{cA}	1,83±0,07^{cD}	ND	49,86±0,45^{cB}	11,91±0,07^{cC}

$^{a-c}$Para la misma muestra (KHA, RTA o TMA) y compuesto, las diferentes letras en las columnas indican diferencias significativas entre los lavados ($p < 0,05$). Para la misma muestra, las diferentes letras X-Y en la misma fila indican diferencias significativas entre los azúcares ($p < 0,05$) y las diferentes letras A–E en la misma fila indican diferencias significativas entre los ácidos orgánicos ($p < 0,05$). KHA1: agua de dátiles en la fase khalal tras el primer lavado; KHA2: agua de dátiles en la fase khalal tras el segundo lavado; KHA3: agua de dátiles en la fase khalal tras el tercer lavado; RTA1: agua de dátiles en la fase rutab tras el primer lavado; RTA2: agua de dátiles en la fase rutab tras el segundo lavado; RTA3: agua de dátiles en la fase rutab tras el tercer lavado; TMA1: agua de dátiles en la fase tamar del primer lavado; TMA2: agua de dátiles en la fase tamar tras el segundo lavado; TMA3: agua de dátiles en la fase tamar tras el tercer lavado, y ND: no detectado.

4.2.3. Caracterización de la harina de dátil

A continuación, en la tabla 16 se muestran los resultados obtenidos tras el estudio de la composición proximal de la harina de los coproductos de dátiles Confitera en tres estados de maduración diferentes (khalal, rutab y tamar).

En los parámetros estudiados en la composición proximal, se observó que, en la humedad, cenizas y el contenido en proteínas no se apreciaron diferencias significativas entre las diferentes harinas ($p < 0,05$). En cambio, los componentes mayoritarios en todas las harinas fueron la FDT (58 – 66%) y el contenido en azúcares totales (19 – 26%), donde la harina TMH mostró los valores más altos en fibra dietéticas y el más bajo en azúcares, siguiendo una tendencia similar a la de las pastas. Las harinas mostraron un bajo contenido en grasas (< 1,2 %) con ligeras diferencias entre las muestras.

En referencia al contenido en minerales, se observó un patrón similar al observado en las pastas de dátil, donde destacan el K, el Mg y el Ca como minerales mayoritarios y el Zn, el Mn, y el Cu como minoritarios. Este contenido se ve afectado por los lavados realizados para la obtención de las harinas, porque, aunque el potasio sigue siendo el mineral principal, al ser el más extraído en el agua de dátiles (tabla 15) su concentración en la harina es menor que en las pastas.
En cuanto al perfil de azúcares, al contrario que con los resultados obtenidos en las pastas de dátil, todas las harinas mostraron un contenido superior de fructosa que de glucosa ($p < 0,05$). El contenido en ácidos orgánicos de las harinas fue inferior al de las pastas, lo que podría deberse a la extracción de los mismos durante los lavados. El ácido málico fue el principal para las tres harinas, en cambio, el ácido cítrico y succínico no se detectaron en TMH, por lo que mostró la acidez más baja ($p < 0,05$).

Tabla 16. Composición proximal y mineral y perfil de azúcares y ácidos orgánicos de la harina de dátil procedente de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	KHH	RTH	TMH
	Composición proximal (g/100g)		
Humedad	4,62±0,33[a]	7,26±1,30[a]	5,69±1,01[a]
Proteínas	7,38±0,30[a]	6,75±0,10[a]	6,78±0,65[a]
Grasa	0,53±0,09[b]	1,07±0,55[a]	1,15±0,73[a]
FDT	60,14±1,98[b]	57,64±1,27[b]	65,98±1,38[a]
Cenizas	1,93±0,33[a]	1,34±0,50[a]	1,66±0,26[a]
Azúcares	25,40±0,26[a]	25,94±0,54[a]	18,74±0,82[b]
	Composición mineral (mg/100g)		
K	395,31±8,88[a]	398,29±9,02[a]	359,36±12,96[b]
Mg	185,85±1,85[a]	164,92±0,24[b]	165,05±6,05[b]
Ca	280,34±5,58[a]	233,39±3,54[b]	272,79±6,85[a]
Na	8,42±0,43[a]	5,31±0,19[c]	6,77±0,56[b]
Fe	3,52±0,08[b]	3,34±0,05[c]	4,24±0,01[a]
Zn	1,37±0,02[a]	1,30±0,03[b]	1,40±0,02[a]
Cu	2,14±0,04[a]	1,18±0,00[c]	1,71±0,02[b]
Mn	1,59±0,05[c]	2,22±0,04[b]	2,45±0,01[a]
	Azúcares (mg/g)		
Glucosa	111,13±3,68[a]	112,68±4,27[a]	76,37±1,08[b]
Fructosa	129,56±2,28[b]	141,35±0,52[a]	94,15±1,35[c]
	Ácidos orgánicos (mg/g)		
Oxálico	0,12±0,00[c]	0,53±0,00[a]	0,36±0,01[b]
Tartárico	ND	0,52±0,03[a]	0,28±0,02[b]
Málico	5,26±0,16[a]	2,38±0,13[b]	1,95±0,04[c]
Cítrico	0,40±0,05[b]	1,15±0,10[a]	ND
Succínico	1,64±0,16[b]	2,19±0,04[a]	ND

[a-c] Para el mismo compuesto, las diferentes letras en la misma fila indican diferencias significativas entre las muestras (etapas de maduración) ($p < 0,05$). Harina de dátiles KHH en la etapa khalal, harina de dátiles RTH en la etapa rutab, harina de dátiles TMH en la etapa tamar y ND: no detectado.

En la tabla 17 se muestran las propiedades fisicoquímicas de las tres harinas estudiadas. En cuanto al pH, todas fueron ligeramente ácidas, siendo TMH la que menor valores de pH mostró ($p < 0,05$). Para las coordenadas de color, la harina disminuyó luminosidad a medida que avanzaba la fase de maduración, siendo la KHF la más clara y la TMF la más oscura ($p < 0,05$). En este caso, el cambio de color más oscuro podría atribuirse al oscurecimiento no enzimático, como las reacciones de Maillard (Hasan et al., 2022) inducido por el tratamiento térmico

durante el procesamiento de las harinas. Este efecto también podría ser responsable del aumento de los valores de tono (h) hacia tonos amarillo-naranja.

Tabla 17. Propiedades fisicoquímicas de la harina de dátil procedente de los coproductos de dátiles del cultivar Confitera en diferentes estados de maduración.

	KHH	RTH	TMH
pH	6,36±0,01[a]	6,32±0,01[b]	6,22±0,03[c]
L*	62,46±0,27[a]	61,97±0,25[a]	56,72±0,13[b]
a*	3,93±0,04[a]	3,15±0,07[b]	3,33±0,09[b]
b*	13,44±0,08[b]	12,96±0,07[c]	13,89±0,12[a]
C*	14,01±0,08[b]	13,34±0,07[c]	14,28±0,14[a]
h	73,71±0,19[b]	76,33±0,28[a]	76,54±0,24[a]

[a-c]Para el mismo parámetro, las diferentes letras en la misma fila indican diferencias significativas entre las muestras (etapas de maduración) ($p < 0,05$). L* luminosidad, a* coordenada rojo/verde, b* coordenada amarillo/azul, C* croma, H* tono. Harina de dátiles KHH en la etapa khalal, harina de dátiles RTH en la etapa rutab, harina de dátiles TMH en la etapa tamar.

4.3. EVALUACIÓN *IN VITRO* DE LAS PROPIEDADES BIOLÓGICAS DE LA HARINA Y EL AGUA DE DÁTIL

Tanto en la figura 16 como en la 17 se detallan las curvas de crecimiento de las seis cepas de *Lactobacillus* y las cinco de *Bifidobacterium* en los diferentes medios de MRS agar (Man, Rogosa y Sharpe agar) con diferentes fuentes de carbono. Como era de esperar, el medio de control negativo (MRS sin glucosa) mostró un crecimiento microbiano significativamente restringido para todas las cepas analizadas, lo que corresponde a una tasa baja de crecimiento, ya que no hay suficiente fuente de carbono disponible para permitir el crecimiento normal de las cepas de *Lactobacillus* y *Bifidobacterium*.

En la figura 16 se muestra el crecimiento de las seis cepas de *Lactobacillus* dependiendo de la fuente de carbono. Se pudo observar que las cepas que mostraron menor tasa de crecimiento fueron *L. acidophilus ki* y *La-5* ($p < 0,05$). Además, la adición de FOS (Fructoologosacáridos) como única fuente de carbono no mejoró el crecimiento de las seis cepas de *Lactobacillus* tanto como la glucosa. El agua de dátil, especialmente a una concentración del 10 %, demostró un aumento del crecimiento más elevado en comparación con el FOS, que además, fue comparable al efecto de la glucosa sobre el crecimiento de todas las cepas de

lactobacilos examinadas, excepto las dos cepas *L. acidophilus Ki* y *La-5*. En este último caso, los parámetros de crecimiento mejoraron tanto en términos de tasas de crecimiento específicas como de niveles de absorbancia. Se observaron variaciones significativas entre las dos concentraciones de agua de dátiles ($p < 0,05$), no obstante, es importante destacar que la incorporación de medios con un 2 % de agua de dátil condujo a un aumento de las tasas de crecimiento en comparación con el uso de FOS para todas las cepas analizadas. En base a lo anterior, cuanto mayor sea el porcentaje de agua de dátil, mayor será la concentración de fuentes de carbohidratos disponibles, es decir, azúcares. Una mayor concentración de azúcares disponibles conduce a tasas de crecimiento más elevadas. Sin embargo, cabe señalar que superar el umbral del 10% de agua de dátil no tiene por qué traducirse necesariamente en mayores tasas de crecimiento, ya que unas concentraciones excesivamente altas de azúcar podrían inhibir el crecimiento bacteriano (Mizzi et al., 2020; Cai et al., 2021).

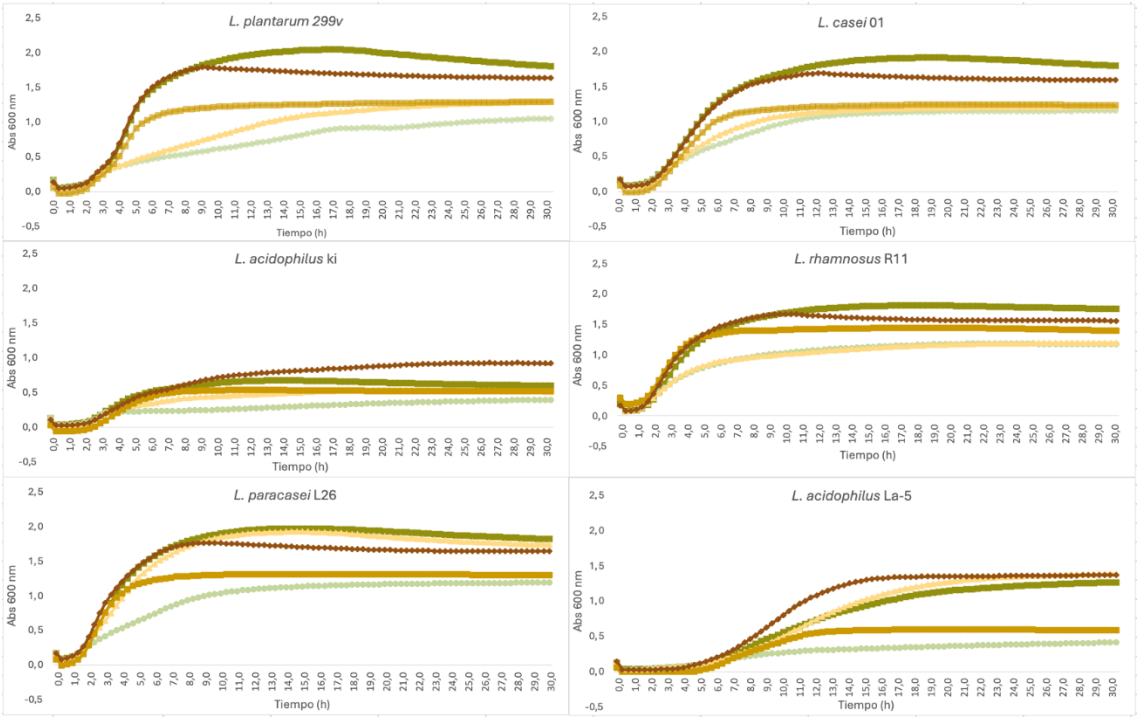


Figura 16. Curvas de crecimiento de cepas de *Lactobacillus* (*L. plantarum 299v, L. acidophilus ki, L. acidophilus La-5, L. paracasei L26, L. casei 01* y *L. rhamnosus R11*) cultivadas en medios de cultivo diferentes (medio MRS basal sin glucosa (verde claro), MRS con glucosa (verde oscuro), MRS con FOS (amarillo), MRS con 2 % de agua de dátil (marrón claro) y MRS con 10 % de agua de dátil (marrón oscuro)), determinadas midiendo la DO de los cultivos a 600 nm durante un período de 30 h.

Para las cepas de *Bifidobacterium* (figura 17) se observó que *B. animalis BLC* mostró la tasa de crecimiento más elevada independientemente de la fuente de carbono, mientras que *B. animalis Bo* mostró los valores más bajos ($p < 0,05$). Excepto *B. animalis Bo*, todas las cepas de *Bifidobacterium* ensayadas mostraron niveles máximos de DO600 nm similares cuando se cultivaron en MRS suplementado con un 10 % de agua de dátil en comparación con la glucosa. Además, el crecimiento en MRS suplementado con un 2% de agua de dátil se asemejó mucho al obtenido con FOS para todas las cepas, menos en *B. animalis Bo*. El agua de dátil se compone principalmente de sólidos solubles en agua que se transfieren durante el procesamiento de los coproductos, siendo estos principalmente glucosa y fructosa, que representan el 38,2-44,7% y el 36,8-40,1% respectivamente de la masa seca total (tabla 14) (Kamal-Eldin et al., 2020), lo que explicaría su alta tasa de crecimiento bacteriano.

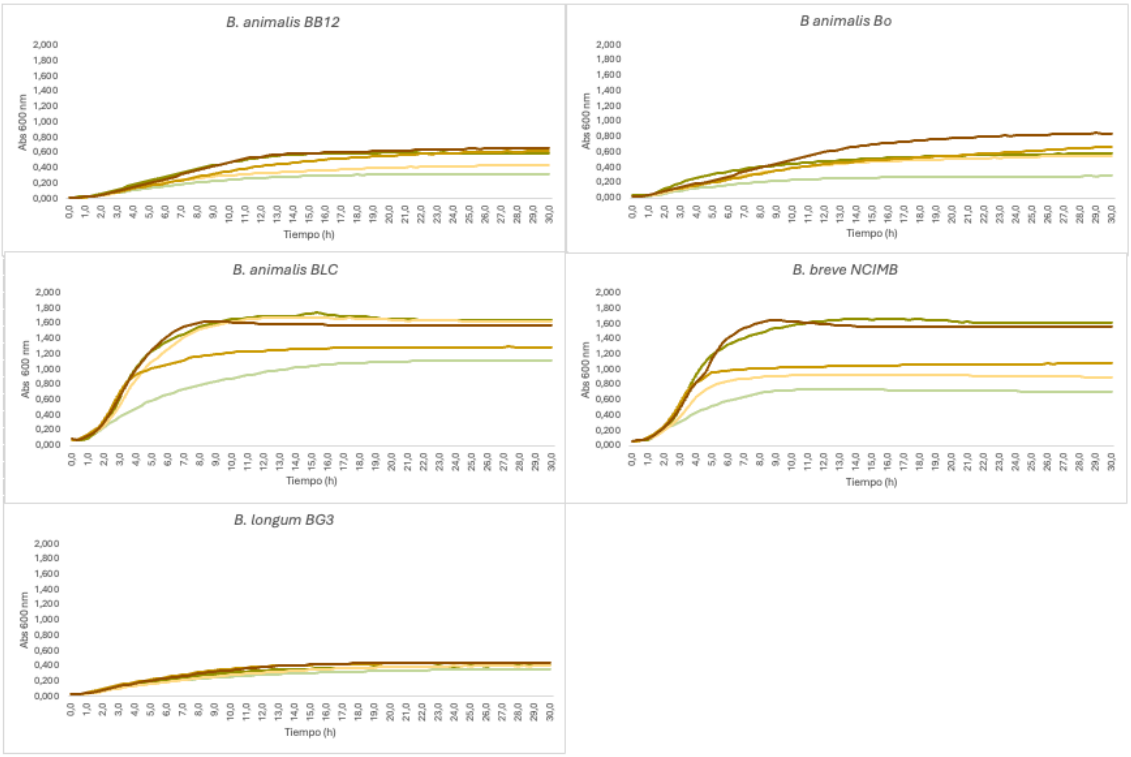


Figura 17. Curvas de crecimiento de cepas de *Bifidobacterium* (*B. animalis BB12, B. animalis Bo, B. animalis BLC, B. breve NCIMB* y *B. longum BG3*) cultivadas en medios de cultivo diferentes (medio MRS basal sin glucosa (verde claro), MRS con glucosa (verde oscuro), MRS con FOS (amarillo), MRS con 2 % de agua de dátil (marrón claro) y MRS con 10 % de agua de dátil (marrón oscuro)), determinadas midiendo la DO de los cultivos a 600 nm durante un período de 30 h.

En la figura 18 se muestran el crecimiento de las cuatro bacterias probióticas seleccionadas (*Lacticaseibacillus rhamnosus* 11, *Lactobacillus casei* 01, *Bifidobacterium breve* NCIMB y *Bifidobacterium animalis* BLC) con el objetivo de evaluar el potencial del factor de crecimiento (tabla 18) en la harina de dátil a dos concentraciones diferentes (2% y 6%) durante 24 horas.

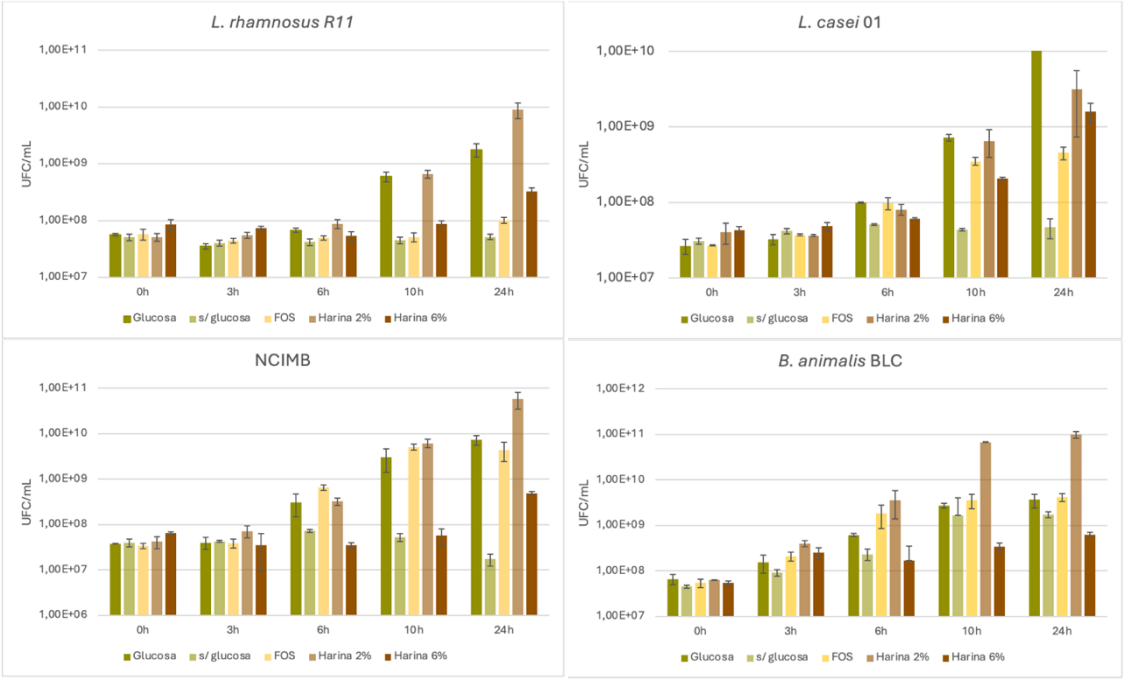


Figura 18. Evaluación del crecimiento de bacterias probióticas seleccionadas (*Lacticaseibacillus rhamnosus* 11, *Bifidobacterium breve* NCIMB, *Lactobacillus casei* 01, *Bifidobacterium animalis* BLC) en diferentes medios de cultivo.

Se puede observar que la harina de dátil (2%) mostró un alto potencial prebiótico ya que presentó un mayor número de células viables (UFC/mL) en comparación con la muestra MRS-FOS (control prebiótico positivo) y MRS-glucosa (control positivo) tras las 24 horas de incubación. Aunque en la bacteria *L.casei* 01 no se dio esta misma situación, el número de células viable en las muestras con harina de dátil (2% y 6%) siguió siendo superior con respecto a MRS-FOS. Además, cabe destacar que para las cepas de *Bifidobacterium* (*B. breve* NCIMB y *B. animalis* BLC) el número general de células viables era bastante superior comparado con *L.casei* 01 y *L. rhamnosus* 11. Como se ha mostrado anteriormente (tabla 16), la harina de dátil destaca por tener altas cantidades de fibra dietética total, por ello se previó que las condiciones que contenían un 2% y un 6% de harina de dátil facilitarían el crecimiento de las cepas probióticas seleccionadas.

Una situación similar se reportó en un estudio sobre el potencial prebiótico de semillas de dátiles o harina de yacón para estimular el crecimiento de cepas de *Lactobacillus* (Sousa et al., 2015; Al-Thubiani y Khan, 2017). Por lo tanto, basándonse en los resultados mencionados anteriormente, la harina de dátil al 2% parece ser la concentración óptima para promover el crecimiento de las cepas probióticas seleccionadas.

Tabla 18. Tasas de crecimiento específicas (h^{-1}) de cepas de *Lactobacillus* y *Bifidobacterium* analizadas en los diferentes medios MRS con diferentes fuentes de carbono, incluyendo dos concentraciones de agua de dátil (WD) (2% y 10%).

	Glucosa	s/glucosa	FOS	WD 2%	WD 10%
Lactobacillus					
L. acidophilus Ki	0,423	0,265	0,400	0,558	0,480
L. acidophilus La-5	0,349	0,203	0,307	0,404	0,437
L. rhamnosus R11	0,752	0,420	0,568	0,607	0,752
L. paracasei L26	0,611	0,339	0,543	0,664	0,650
L. casei 01	0,601	0,391	0,515	0,621	0,627
L. plantarum 299v	0,833	0,447	0,556	0,754	0,838
Bifidobacterium					
B. animalis BB12	0,596	0,483	0,572	0,523	0,568
B. animalis Bo	0,421	0,403	0,441	0,324	0,394
B. animalis BLC	0,715	0,578	0,604	0,647	0,690
B. breve NCIMB	0,679	0,419	0,616	0,631	0,655
B. longum BG3	0,449	0,429	0,456	0,466	0,472

Además, en la figura 19 se observan los resultados de la evolución del pH durante el crecimiento de las cuatro bacterias probióticas seleccionadas durante las 24 horas de incubación, los cuales afirman los resultados comentados anteriormente en la figura 18. Como se esperaba, los niveles de pH mostraron una disminución durante el período de incubación, lo que indica la actividad fermentativa de las bacterias probióticas. Esta disminución puede atribuirse a la conversión de los azúcares disponibles en los ácidos correspondientes a través de la actividad fermentativa; el crecimiento y la actividad de acidificación estaban bien correlacionados. Por esto, se puede concluir razonablemente que la harina de dátil

adicionada a un 2% ejerce un efecto prebiótico sobre las cepas probióticas seleccionadas.

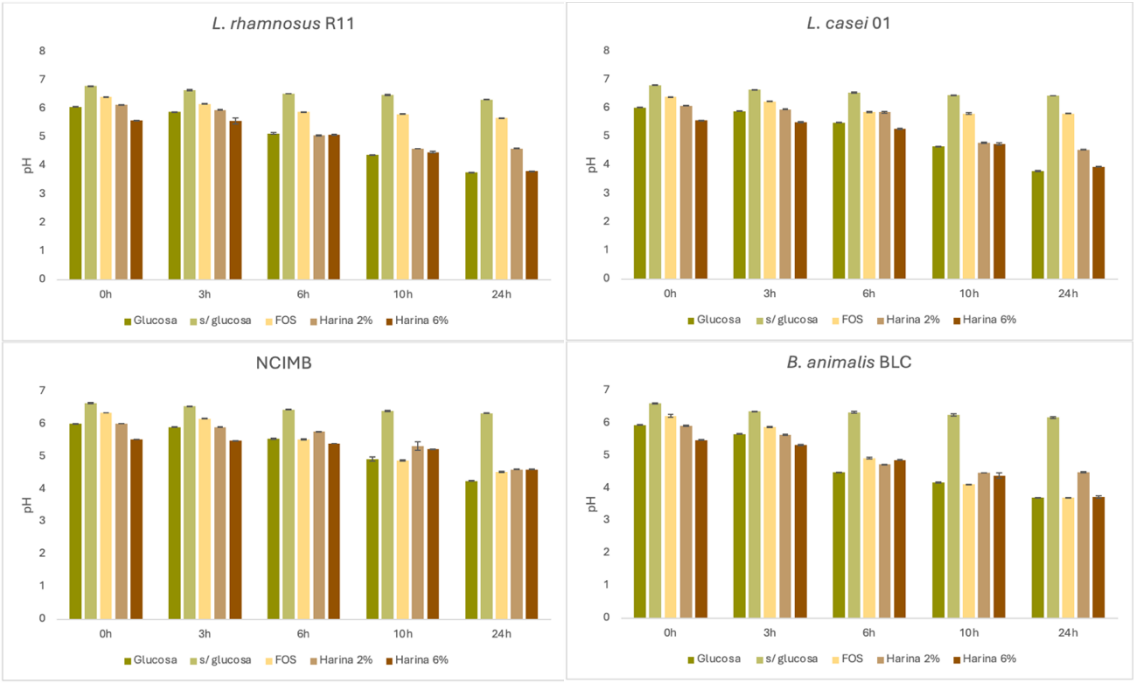


Figura 19. Evolución del pH durante el crecimiento de bacterias probióticas seleccionadas (*Lacticaseibacillus rhamnosus* 11, *Bifidobacterium breve* NCIMB, *Lactobacillus casei* 01, *Bifidobacterium animalis* BLC) en diferentes medios de cultivo.

La diabetes es un trastorno metabólico crónico caracterizado por niveles elevados de glucosa en sangre, la cual provoca daños a largo plazo en diversos órganos, siendo la responsable directa de 1,5 millones de muertes al año, de acuerdo con la Organización Mundial de la Salud (OMS, 2024). La enzima α-glicosidasa se encuentra en el intestino delgado, más concretamente en las microvellosidades, la cual transforma los oligosacáridos y los disacáridos en monosacáridos, lo que facilita la absorción de carbohidratos y eleva los niveles de azúcar en sangre. Los inhibidores de la α-glicosidasa pueden retrasar la absorción de carbohidratos impidiendo la hidrólisis de los disacáridos y la absorción de glucosa (Vichayanrat et al., 2002). La búsqueda de fuentes naturales de inhibidores de la α-glicosidasa ha atraído la atención de la comunidad científica, ya que, varios autores han descrito las propiedades antidiabéticas de los extractos de frutas y verduras, entre ellos el kiwi, la pulpa de limón, la cáscara de limón, la pera, la cebolla roja y el tomate, entre otros (Wu et al., 2015). Además, la actividad

antidiabética se relacionó positivamente con el contenido de polifenoles. En la tabla 19 se muestra la capacidad inhibitoria de los dos productos alimentarios intermedios estudiados (agua de dátil y harina de dátil). En ella se puede observar que la harina de dátil (87,62%) mostró una mayor actividad inhibitoria de la α-glucosidasa que el agua de dátil (76,34%) ($p < 0,05$), lo que estaría dentro de lo esperado puesto que la harina mostró un mayor contenido en compuestos fenólicos que el agua de dátil (tabla 19). Además, se ha demostrado que el dátil contiene compuestos bioactivos no fenólicos solubles en agua que tienen actividad contra la enzima α-glucosidasa (Khan et al., 2016; Mia et al., 2020). Esto concuerda con lo obtenido en estudios similares en los que se reporta que varios extractos de dátiles demostraron una actividad inhibidora de la α-glucosidasa, incluso mayor que la observada contra la α-amilasa (El Abed et al., 2017). Es cierto que este estudio se realizó *in vivo*, demostrando que la administración a corto plazo (ingesta durante un mes) da lugar a una reducción de los niveles de glucosa en sangre y a un aumento de la concentración de insulina a través de mecanismos como el aumento del número de células β, la estimulación de la secreción de insulina y la disminución del vaciado gástrico por la acción de los polifenoles (Evans et al., 2018; Mia et al., 2020).

Tabla 19. Actividad antidiabética (inhibición de la α-glucosidasa) y actividad antihipertensiva (inhibición de la enzima convertidora de angiotensina I (ECA)) de agua y harina de dátil.

	Agua de dátil	**Harina de dátil**
Inhibición α-glucosidasa (%)	76,34±0,78[b]	87,62±2,04[a]
Inhibición ECA (%)	55,73±4,92[b]	92,24±0,73[a]

[a-b]Para el mismo parámetro, las diferentes letras en la misma fila indican diferencias significativas entre las muestras ($p < 0,05$).

La hipertensión es uno de los principales factores de riesgo de enfermedades cardiovasculares, la cual puede llegar a causar morbilidad y mortalidad y afecta a uno de cada tres adultos en el mundo (OMS, 2023). Además, es aproximadamente dos veces más frecuente en personas con diabetes que en personas sin diabetes, lo que la convierte en un importante problema de salud pública (Farida et al., 2023). La supresión de la enzima convertidora de angiotensina I (ECA) es una estrategia crucial para controlar la hipertensión, una crisis sanitaria

mundial de proporciones epidémicas y un factor importante que contribuye al riesgo de enfermedades cardiovasculares (Faustino et al., 2023; OMS, 2023). Es cierto que los cambios en el estilo de vida, incluida la dieta, han despertado recientemente interés debido a los efectos secundarios indeseables asociados con los inhibidores sintéticos de la ECA. Por ello, se ha propuesto el consumo de frutas y verduras, ricas en vitaminas, minerales y compuestos bioactivos, como factor de prevención relevante. Además, se están investigando las frutas y verduras como posibles fuentes de compuestos naturales con propiedades antihipertensivas (Yousefi et al., 2021). Los dátiles contienen diversos compuestos bioactivos con posibles beneficios para la salud, por lo que la actividad antihipertensiva de estos puede deberse principalmente a la presencia de flavonoides, minerales, vitaminas y fibras (Yousefi et al., 2021).

En la tabla 19 se puede observar que la harina de dátil mostró una mayor actividad inhibidora de la ECA, cercana al 100% (92,24%), que el agua de dátil (55,73%). Los dátiles contienen varios compuestos bioactivos que regulan y controlan la hipertensión, entre ellos el ácido láurico, el ácido linolénico, el ácido palmítico, los tocoferoles, el β-sitosterol y la isosorbida (Obode et al., 2020). La harina de dátil demostró una mayor inhibición de la ECA que el agua de dátil, ya que los compuestos bioactivos se encuentran principalmente en la pulpa, no siempre son solubles en agua y, por lo tanto, no se transfieren al agua de dátil. Varios informes han demostrado que los dátiles tienen un potente efecto antihipertensivo debido a su capacidad para inhibir la enzima convertidora de angiotensina (Vayalil, 2012; Al-Dashti et al., 2021; Fernández-López et al., 2022).

4.4. DESARROLLO Y CARACTERIZACIÓN DE PRODUCTOS LÁCTEOS FORTIFICADOS CON PRODUCTOS ALIMENTARIOS INTERMEDIOS PROCEDENTES DE COPRODUCTOS DEL DATIL CONFITERA

4.4.1. Yogures elaborados con leche de cabra y fortificados con pasta y harina de dátil confitera

La incorporación de pasta de dátil (3% y 6%) y harina de dátil (3% y 6%) a yogures de leche de cabra no alteró el proceso de formación del yogur (curva de acidificación), resultando un proceso tecnológicamente viable (figura 20).

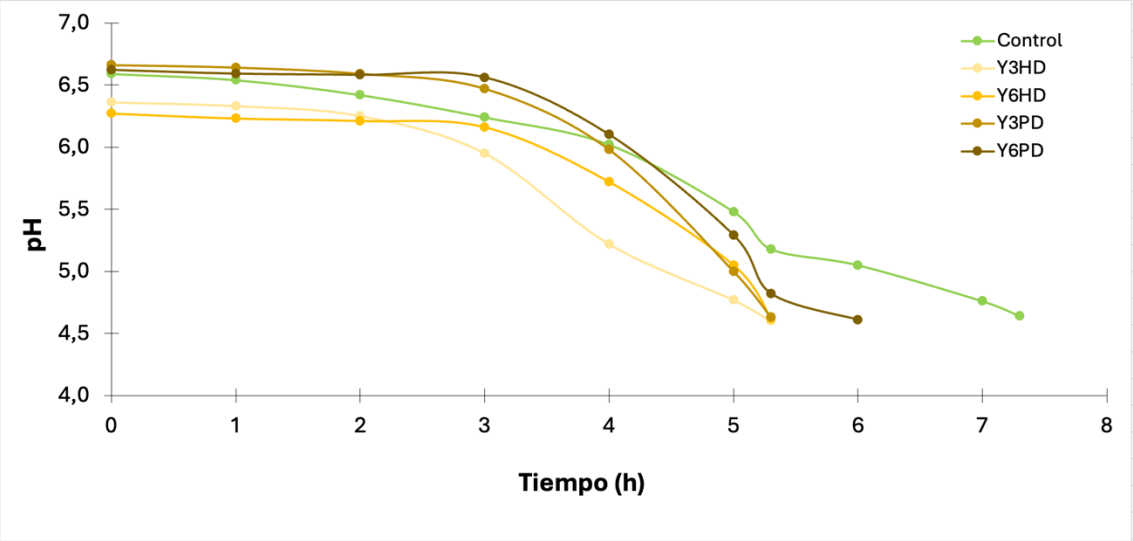


Figura 20. Curva de acidificación de los yogures de cabra fortificados con pasta y harina de dátil Confitera.

En la tabla 20 se muestran los resultados de la composición proximal de los diferentes yogures de cabra elaborados con pasta y harina de dátil. Relacionado con la humedad, se pudo observar que la adición de harina disminuyó el contenido de humedad, siendo 89,91% en el yogur con un 3% de harina y 87,91% en el yogur con un 6% de harina. Por otro lado, la adición de pasta no afectó al contenido en humedad en comparación con la muestra control ($p > 0,05$). Además, tanto las muestras con harina como con pasta mostraron un contenido en grasa mayor que el control ($p < 0,05$), teniendo los valores más elevados las muestras con pasta de dátil (Y3PD: 2,16% y Y6PD: 2,19%). En cuanto al contenido en proteínas y cenizas, no se apreciaron diferencias significativas entre los yogures reformulados y el

control (p > 0,05). Cabe destacar que estas pequeñas diferencias se deben principalmente a la composición de los productos de dátiles añadidos, en este caso pasta y harina de dátil (tabla 12 y 16).

Tabla 20. Composición nutricional de los yogures de cabra fortificados con pasta y harina de dátil Confitera.

	CONTROL	Y3HD	Y6HD	Y3PD	Y6PD
Composición proximal (%)					
Humedad	90,98±0,35^{a}	89,91±0,20^{b}	87,91±0,22^{c}	90,72±0,51^{a}	89,95±0,56^{ab}
Proteínas	2,57±0,01^{a}	2,69±0,10^{a}	2,77±0,01^{a}	2,51±0,04^{a}	2,56±0,14^{a}
Grasa	1,92±0,03^{b}	2,04±0,03^{ab}	2,07±0,01^{ab}	2,16±0,08^{a}	2,19±0,01^{a}
Cenizas	0,89±0,01^{a}	0,95±0,02^{a}	0,98±0,05^{a}	0,90±0,03^{a}	0,93±0,01^{a}
SST	2,56±0,06^{b}	3,77±0,07^{ab}	4,11±0,66^{a}	3,03±0,07^{ab}	3,74±0,23^{ab}

$^{a-c}$ Las diferentes letras en la misma fila indican diferencias significativas (p < 0,05) entre las muestras elaboradas. Y3HD: yogur con 3% de harina de dátil; Y6HD: yogur con 6% de harina de dátil; Y3PD: yogur con 3% de pasta de dátil; Y6PD: yogur con 6% de pasta de dátil. SST: sólidos solubles totales.

En la tabla 21, se observa la concentración de azúcares y ácidos orgánicos de los yogures enriquecidos con PAI (productos alimentarios intermedios) procedentes del dátil (pasta y harina) a tiempo inicial (día 0) y tiempo final (día 21) del almacenamiento. El principal azúcar detectado fue la lactosa. A esta la siguieron la galactosa y la glucosa, esta última solo fue detectada en los yogures reformulados (con pasta o harina de dátil). Las muestras con pasta de dátil mostraron menor cantidad de glucosa que las muestras con harina, además, como era de esperar, a mayor concentración de la pasta de dátil, mayor contenido de glucosa (p < 0,05). Por otro lado, la lactosa disminuyó en todas las muestras durante el almacenamiento (p < 0,05). Además, no se apreciaron diferencias significativas en la concentración de galactosa durante el tiempo de almacenamiento, en ninguno de los yogures analizados (p > 0,05), lo que concuerda con los resultados reportados por otros autores (Trigueros et al., 2011; Bertolino et al., 2015). La concentración de lactosa y galactosa en todos los yogures osciló entre 25,23 y 45,17 mg/g y 18,42-25,73 mg/g, respectivamente, lo que se ajusta a los valores normales de los yogures naturales de leche de cabra (Wang et al., 2023a).

En cuanto a los ácidos orgánicos detectados, el principal fue el ácido láctico, el cual mostró un aumento significativo durante el almacenamiento en todos los yogures elaborados ($p < 0,05$). Aun así, el aumento más significativo se observó en las muestras con pasta de dátil (Y3PD y Y6PD), donde se obtuvo mayor contenido de ácido láctico, tanto al inicio como al final del almacenamiento, con respecto al resto de muestras ($p < 0,05$). Los mayores aumentos en el contenido de ácido láctico se observaron en los yogures con el mayor número de bacterias lácticas iniciadoras viables. Se ha detectado un comportamiento similar en los yogures a los que se les añadieron extractos de frutas que potenciaron el crecimiento de las bacterias iniciadoras (Bertolino et al., 2015; Sendra et al., 2008). Por otro lado, en referencia al ácido cítrico, se observaron pequeñas variaciones entre las muestras debido a la adición de los PAI (harina o dátil), mientras que no se observaron diferencias significativas relacionadas con el tiempo de almacenamiento ($p > 0,05$), lo que concuerda con lo reportado por otros autores (Bertolino et al., 2015).

En la tabla 22 se muestran los resultados de las propiedades fisicoquímicas (pH, acidez, Aw y sinéresis) evaluadas en los yogures elaborados. En general, la incorporación de estos PAI procedentes de coproductos del dátil (harina y pasta) afectó a las propiedades fisicoquímicas de forma e intensidad diferentes durante el almacenamiento en refrigeración. Tanto el pH como la acidez de los yogures se vieron afectados por la adición de estos PAI y por el tiempo de almacenamiento ($p < 0,05$). Las cuatro formulaciones con dátil mostraron un pH más bajo durante todo el almacenamiento, con respecto a la muestra control ($p < 0,05$). Sin embargo, a los 21 días de almacenamiento el pH aumentó en las muestras con adición de dátil, siendo menos significativo en la muestra Y3PD mientras que las muestras con harina de dátil (Y3HD y Y6HD) mostraron un mayor aumento.

Tabla 21. Concentraciones de azúcares y ácidos orgánicos (mg/g) de los yogures de cabra fortificados con pasta y harina de dátil Confitera durante 21de almacenamiento.

			CONTROL	Y3HD	Y6HD	Y3PD	Y6PD
Ácidos orgánicos	Ácido cítrico	0	4,99±0,02^{bA}	5,14±0,18^{aA}	4,74±0,10^{bA}	4,95±0,16^{bA}	4,19±0,19^{cA}
		21	5,07±0,11A^{a}	5,15±0,06^{aA}	4,78±0,02^{abA}	4,91±0,13^{aA}	4,09±0,15^{cA}
	Ácido láctico	0	18,74±0,10^{cB}	19,50±0,14^{bB}	21,87±0,21^{aB}	17,71±0,13^{dB}	17,65±0,11^{dB}
		21	20,41±0,23^{cA}	21,73±0,62^{bA}	25,64±0,08^{aA}	20,82±0,60^{cA}	21,42±0,18^{bA}
Azúcares	Lactosa	0	36,17±0,20^{cA}	45,17±2,39^{aA}	39,91±1,38^{bA}	40,16±2,08^{bA}	45,08±3,52^{aA}
		21	31,44±0,57^{cB}	33,37±0,64^{bB}	36,63±0,78^{aB}	27,25±1,67^{dB}	25,23±0,90^{eB}
	Galactosa	0	20,45±0,28^{bA}	23,65±1,13^{aA}	24,82±0,89^{aA}	18,42±0,93^{bA}	25,73±2,31^{aA}
		21	20,89±0,75^{cA}	24,66±0,10^{bA}	25,29±0,15^{aA}	19,76±0,32^{cA}	24,57±0,20^{bA}
	Glucosa	0	ND	12,79±0,15^{cA}	17,83±0,50^{aA}	9,83±0,52^{dA}	15,21±0,18^{bA}
		21	ND	12,03±0,47^{aA}	17,52±0,75^{aA}	9,33±0,19^{dA}	14,55±0,42^{cB}

$^{a-d}$ Para el mismo compuesto y tiempo de almacenamiento, las diferentes letras minúsculas en la misma columna indican diferencias significativas ($p < 0,05$) debidas a la adición de PAIs del dátil. $^{A-B}$ Para el mismo compuesto y muestra de yogur, las diferentes letras mayúsculas en la misma columna indican diferencias significativas ($p < 0,05$) debidas al tiempo de almacenamiento. ND: no detectado. Y3HD: yogur con 3% de harina de dátil; Y6HD: yogur con 6% de harina de dátil; Y3PD: yogur con 3% de pasta de dátil; Y6PD: yogur con 6% de pasta de dátil.

En cuanto a la acidez, la situación fue a la inversa, los yogures con dátil mostraron una acidez mayor que el yogur control, también durante todo el tiempo de almacenamiento. Este aumento de acidez en las muestras con dátil podría deberse a las actividades metabólicas adicionales provocadas por los cultivos iniciadores durante el almacenamiento, que hidrolizan la lactosa en ácido láctico y la fibra dietética (de los PAI de los dátiles) en ácidos urónicos, lo que da lugar a una disminución de los valores de pH y a un aumento de la acidez durante el almacenamiento (Gaspar et al., 2013; Almusallam et al., 2021). Además, la acidez no está esencialmente relacionada con el crecimiento bacteriano, ya que, durante la refrigeración, las bacterias continúan degradando la lactosa, aunque a un ritmo mucho más lento que a la temperatura óptima para las bacterias termófilas (Brodziak et al., 2020). La acidez de todas las muestras evaluadas (91 – 125 ºD) se encontró dentro del rango mínimo recomendado (60 – 150 ºD) por la Comisión del Codex Alimentarius (Codex, 2015). Esto concuerda con otros estudios de yogures enriquecidos con extractos naturales (Jung et al., 2016; Feng et al., 2019; Almusallam et al., 2021; Basiony et al., 2023).

La cantidad de agua disponible que contienen los alimentos se representa mediante la actividad de agua (Aw). Este parámetro se vio afectado por la adición de PAI de coproductos de dátiles ($p < 0,05$), pero no por el tiempo de almacenamiento ($p > 0,05$). A día 0, las muestras Y6HD mostraron valores más bajos que el resto, lo cual estaría relacionado con la alta capacidad de retención de agua de la harina y su contenido en azúcares (tabla 16). Al final del almacenamiento, todos los yogures con adición de los PAI de dátil mostraron valores de Aw más bajos que el control ($p < 0,05$). Los valores de actividad del agua de todas las muestras durante el tiempo de almacenamiento se situaron en el rango de los valores descritos como normales para los yogures de cabra (0,97-0,98). En referencia a diversos estudios, otros autores han observado que los valores de Aw en los yogures aumentaban significativamente con el tiempo de almacenamiento (Brodziak et al., 2020), mientras que otros autores informaron que la Aw se mantuvo constante (Dos Santos et al., 2018).

Tabla 22. Propiedades fisicoquímicas (pH, acidez, Aw y sinéresis) de yogures de cabra fortificados con harina y pasta de dátil Confitera.

		CONTROL	Y3HD	Y6HD	Y3PD	Y6PD
pH	0	4,67±0,05^{aA}	4,38±0,01^{bA}	4,39±0,01^{bA}	4,25±0,02^{cAB}	4,32±0,01^{bA}
	7	4,54±0,04^{aB}	4,27±0,01^{bB}	4,21±0,02^{bC}	4,20±0,01^{bB}	4,24±0,03^{bB}
	14	4,51±0,01^{aB}	4,26±0,01^{cB}	4,22±0,01^{cC}	4,26±0,03^{cAB}	4,33±0,01^{bA}
	21	4,53±0,02^{aB}	4,39±0,03^{bA}	4,33±0,03^{bB}	4,33±0,06^{bA}	4,37±0,05^{bA}
Acidez (ºD)	0	91,50±0,71^{bB}	96,50±2,12^{bC}	103,50±2,12^{aB}	104,50±0,71^{a} C	105,50±0,71^{aC}
	7	96,50±0,71^{cB}	105,50±0,71^{bB}	108,50±0,71^{bB}	113,00±1,41^{a} B	115,00±0,00^{aB}
	14	105,00±2,83^{bA}	115,50±0,71^{aA}	119,00±1,41^{aB}	114,50±0,71^{a} C	119,50±0,71^{aA}
	21	106,00±1,41^{bA}	119,00±1,41^{aA}	122,50±0,71^{aA}	118,50±2,12^{a} A	122,00±1,41^{aA}
Aw	0	0,972±0,001^{aA}	0,973±0,001^{aA}	0,967±0,001^{bA}	0,972±0,001^{a} A	0,972±0,000^{aA}
	7	0,973±0,001^{aA}	0,972±0,001^{aA}	0,971±0,001^{aA}	0,972±0,000^{a} A	0,971±0,000^{aA}
	14	0,974±0,000^{aA}	0,972±0,001^{aA}	0,973±0,001^{aA}	0,972±0,003^{a} A	0,973±0,001^{aA}
	21	0,977±0,001^{aA}	0,973±0,001^{bA}	0,971±0,000^{bA}	0,972±0,001^{b} A	0,973±0,000^{bA}
Sinéresis (%)	0	ND	0,27±0,07^{bD}	0,48±0,02^{aD}	ND	ND
	7	0,10±0,02^{cC}	0,50±0,02^{bC}	1,29±0,02^{aBC}	ND	ND
	14	0,21±0,03^{cB}	0,85±0,05^{bB}	1,14±0,13^{aC}	ND	ND
	21	0,43±0,03^{cA}	1,05±0,05^{bA}	1,43±0,08^{aA}	0,11±0,03^{eA}	0,25±0,05^{dA}

$^{a-d}$ Las diferentes letras minúsculas en la misma fila indican diferencias significativas ($p < 0,05$) debido a la adición de pasta y harina de dátil. $^{A-D}$ Las diferentes letras mayúsculas en la misma columna indican diferencias significativas ($p > 0,05$) debido al tiempo de almacenamiento. ND: no detectado. Y3HD: yogur con 3% de harina de dátil; Y6HD: yogur con 6% de harina de dátil; Y3PD: yogur con 3% de pasta de dátil; Y6PD: yogur con 6% de pasta de dátil.

La sinéresis es un factor físico importante ya que afecta a la estabilidad y aceptación del yogur, por lo tanto, a menor sinéresis mayor estabilidad (Kiros et al., 2016). La sinéresis se vio afectada tanto por la adición de los PAI del dátil como por el tiempo de almacenamiento ($p < 0,05$). Los yogures a los que se adicionó harina de dátil mostraron la sinéresis más alta durante el periodo de almacenamiento, siendo estos valores mayores a concentraciones más altas de harina ($p < 0,05$). Por el contrario, en los yogures con pasta de dátil (Y3PD y Y6PD) solo se detectó sinéresis al final del almacenamiento (día 21), siendo estos los valores más bajos (Y3PD: 0,11% y Y6PD: 0,25%) en comparación con el resto de las muestras ($p < 0,05$). Mientras, el yogur control, no mostró sinéresis a tiempo 0, pero esta fue

aumentando progresivamente durante el tiempo de almacenamiento ($p < 0,05$). Estos resultados demuestran que la pasta de dátil puede reducir los cambios de sinéresis durante el almacenamiento en frío de los yogures de leche de cabra y, por lo tanto, mantener sus atributos de calidad física. Además, se ha descrito un comportamiento similar en yogures a los que se les ha añadido orujo o puré de frutas (Jung et al., 2016; Feng et al., 2019; Kwon et al., 2019; Almusallam et al., 2021; Rashwan et al., 2024), lo que se atribuye a su alta capacidad de retención de agua (CRA) causada por las moléculas de polisacáridos que pueden absorber una gran cantidad de agua y mejorar la rigidez de la red de gel proteica (Du et al., 2023; Fan et al., 2023). Hay que tener en cuenta también que algunos compuestos fenólicos presentes en estos extractos de fruta y en los PAI obtenidos de coproductos del dátil podrían afectar a la red de coagulación de las proteínas y, por lo tanto, a la sinéresis (Durmus et al., 2021).

El color es un parámetro de calidad muy importante que influye en gran medida en la comercialización y aceptación de los alimentos por parte de los consumidores. En la tabla 23 se muestran los valores obtenidos tras la evaluación de las coordenadas CIELab*, croma, tono e índice de blancura (IB) de los yogures. La adición de PAI del dátil a los yogures provocó modificaciones estadísticamente significativas ($p < 0,05$) con respecto al yogur control en todas las propiedades cromáticas analizadas. Las muestras enriquecidas con dátil mostraron menor luminosidad (L*), tono (h*) e IB y mayor saturación del color (C*) y valores de a* y b* con respecto al control durante el almacenamiento. Estos cambios de color estarían relacionados con el color marrón anaranjado de los PAI añadidos, debido a su contenido en carotenos, pigmentos de color amarillo anaranjado a rojo y pigmentos de melanina (Alam et al., 2022). Cabe destacar que en las muestras con harina de dátil (Y3HD y Y6HD) los cambios de color fueron más pronunciados que en el resto de las muestras. Algunos autores informaron de una situación similar en yogures enriquecidos con extractos naturales (Pelaes Vital et al., 2015; Kwon et al., 2019). En cuanto al tono, se apreció un cambio de color debido al PAI adicionado; los yogures pasaron de un color amarillo-limón (control) a un amarillo-anaranjado (yogures con pasta o harina de dátil).

Tabla 23. Propiedades fisicoquímicas (L*, a*, b*, C*, h* e IB) de yogures de cabra fortificados con harina y pasta de dátil Confitera.

		CONTROL	Y3HD	Y6HD	Y3PD	Y6PD
L*	0	89,39±0,34^{aA}	77,35±0,61^{dAB}	75,23±0,37^{dA}	86,95±0,13^{bB}	84,87±0,10^{cA}
	7	89,57±0,08^{aA}	79,48±0,45^{cA}	73,08±0,37^{dBC}	85,03±0,79^{bB}	84,60±0,35^{bA}
	14	90,11±0,24^{aA}	77,95±1,45^{dA}	74,57±0,96^{eAB}	87,24±0,85^{bA}	83,41±0,29^{cB}
	21	89,83±0,26^{aA}	74,59±1,47^{dB}	72,22±1,10^{eC}	86,38±0,39^{bAB}	82,28±0,42^{cC}
a*	0	-1,32±0,01^{eA}	2,67±0,07^{bB}	3,11±0,11^{aB}	-0,45±0,09^{dC}	0,34±0,02^{cB}
	7	-1,39±0,04^{dA}	2,06±0,11^{bB}	3,23±0,06^{aB}	0,31±0,14^{cA}	0,30±0,10^{cB}
	14	-1,31±0,05^{dA}	2,44±0,31^{aB}	2,97±0,21^{aB}	-0,65±0,08^{cC}	0,70±0,15^{bA}
	21	-1,36±0,03^{dA}	3,57±0,59^{aA}	4,04±0,43^{aA}	-0,09±0,03^{cB}	0,86±0,13^{bA}
b*	0	6,04±0,06^{dA}	11,12±0,25^{bA}	12,49±0,44^{aA}	6,27±0,26^{dB}	8,15±0,18^{cAB}
	7	6,09±0,17^{cA}	8.17±0,29^{bB}	11,38±0,68^{aA}	7,38±0,24^{bA}	7,83±0,21^{bB}
	14	6,27±0,03^{dA}	9,95±0,11^{bA}	11,36±0,87^{aA}	6,74±0,22^{dB}	8,60±0,61^{cAB}
	21	6,17±0,07^{dA}	11,13±1,11^{aA}	13,20±1,14^{aA}	7,16±0,24^{cA}	9,02±0,49^{bA}
C*	0	6,18±0,06^{dA}	11,44±0,26^{bA}	12,87±0,45^{aA}	6,28±0,26^{dB}	8,15±0,19^{cB}
	7	6,24±0,16^{dA}	8,43±0,31^{bB}	11,83±0,65^{aA}	7,38±0,25^{cA}	7,83±0,20^{bcBC}
	14	6,41±0,03^{dA}	10,25±0,15^{bA}	11,75±0,82^{aA}	6,77±0,22^{dB}	8,63±0,61^{cAB}
	21	6,32±0,06^{dA}	11,69±1,23^{bA}	13,80±1,21^{aA}	7,16±0,24^{dA}	9,06±0,50^{cA}
h*	0	102,34±0,19^{aA}	76,47±0,14^{dA}	76,04±0,16^{dA}	94,08±0,91^{bA}	87,60±0,17^{cA}
	7	102,82±0,37^{aA}	75,86±0,34^{cA}	74,09±0,84^{cAB}	87,57±0,99^{bB}	87,78±0,80^{bA}
	14	101,85±0,14^{aA}	76,24±1,66^{dA}	75,27±1,81^{dAB}	95,52±0,80^{bA}	85,40±0,77^{cB}
	21	102,44±0,42^{aA}	72,28±1,41^{dB}	72,97±0,33^{dB}	90,77±0,30^{bB}	84,56±0,50^{cB}
IB	0	87,72±0,26^{aA}	74,63±0,56^{dB}	72,09±0,31^{eA}	85,51±0,10^{bA}	82,82±0,17^{cA}
	7	87,85±0,12^{aA}	77,81±0,53^{cA}	70,59±0,20^{dAB}	83,30±0,80^{bB}	82,72±0,23^{bA}
	14	88,21±0,19^{aA}	75,68±1,37^{dAB}	71,98±0,68^{eA}	85,55±0,68^{bA}	81,29±0,14^{cB}
	21	88,03±0,19^{aA}	72,03±1,85^{dC}	68,98±1,50^{eB}	84,61±0,44^{bAB}	80,09±0,57^{cC}

$^{a-e}$ Las diferentes letras minúsculas en la misma fila indican diferencias significativas ($p < 0,05$) debido a la adición de pasta y harina de dátil. $^{A-D}$ Las diferentes letras mayúsculas en la misma columna indican diferencias significativas ($p > 0,05$) debido al tiempo de almacenamiento. ND: no detectado. Y3HD: yogur con 3% de harina de dátil; Y6HD: yogur con 6% de harina de dátil; Y3PD: yogur con 3% de pasta de dátil; Y6PD: yogur con 6% de pasta de dátil.

En cualquier caso, estos cambios durante el almacenamiento podrían estar relacionados con el desarrollo de la acidez y las modificaciones de la estructura tridimensional del yogur, la cual favorece la liberación de estos pigmentos de los dátiles de la matriz del yogur, haciéndolos más sensibles a las degradaciones o transformaciones químicas, enzimáticas y microbianas. Además, se han descrito cambios de color relevantes durante el almacenamiento en frío en yogures con extractos de frutos rojos, que se atribuyeron a los cambios en el color de algunos

compuestos bioactivos (por ejemplo, antocianinas o betalaínas) causados por la disminución del pH (Durmus et al., 2021; Basiony et al., 2023; Rashwan et al., 2024).

En cuanto a la textura, en general, la adición de PAI procedentes de los coproductos del dátil aumentó la firmeza, la consistencia y la cohesión del yogur, y disminuyó el índice de viscosidad, siendo este efecto mayor en el yogur al que se le adicionó harina de dátil ($p < 0,05$) (tabla 24). A día 0 y 21, los yogures con harina de dátil mostraron una mayor firmeza y consistencia, pero un índice de viscosidad menor que el resto de los yogures. Se podría decir que la adición de harina de dátiles produjo modificaciones de textura más fuertes en los yogures que la adición de pasta de dátiles. La firmeza y la consistencia de los yogures se han relacionado anteriormente con el contenido sólidos totales, que proporciona una estructura consistente y estable al yogur (Wang et al., 2023a) incluyendo compuestos fenólicos y polisacáridos (Rashwan et al., 2022; Rashwan et al., 2024). Además, varios autores han informado de una mejora en la textura del yogur debido a la adición de extractos de frutas (Du et al., 2023; Fan et al., 2023; Rashwan et al., 2024).

En las figuras 21 y 22 se muestra el recuento de las bacterias lácticas del cultivo iniciador (*Lactobacillus* spp. y *Streptococcus* spp.) tanto de los yogures control como de los yogures con adición de PAI (pasta y harina). La adición de productos alimentarios intermedios de dátil al yogur no afectó a la supervivencia de las cepas iniciadoras, ya que, tras 21 días de almacenamiento, el número de células viables de ambas cepas en todos los yogures era superior al mínimo exigido legalmente en la elaboración de yogur por el Codex Alimentarius (107 UFC/g) (Codex, 2015). En la figura 21 se observa que el número de células viables de *Lactobacillus* spp. en los yogures con adición de pasta de dátil alcanzaron un valor medio superior (8,92 log10 UFC/g) al resto de muestras (control: 8,33 log10 UFC/g; harina de dátil: 8,88 log10 UFC/g). Una situación similar a la anterior ocurrió en las células de *Streptococcus* spp. (figura 22).

Tabla 24. Propiedades texturales de yogures de cabra fortificados con harina y pasta de dátil Confitera.

		CONTROL	Y3HD	Y6HD	Y3PD	Y6PD
Firmeza (N)	0	0,06±0,01^{ab}	0,11±0,01^{bAB}	0,10±0,02^{bAB}	0,05±0,01^{aA}	0,06±0,03^{aB}
	7	0,06±0,00^{a}	0,10±0,01^{bcAB}	0,12±0,02^{cB}	0,07±0,02^{abB}	0,07±0,02^{abB}
	14	0,05±0,01^{a}	0,08±0,01^{abA}	0,10±0,01^{bA}	0,06±0,01^{abB}	0,08±0,03^{abB}
	21	0,05±0,01^{a}	0,12±0,02^{bB}	0,09±0,01^{bA}	0,04±0,01^{aA}	0,05±0,01^{aA}
Consistencia (N.s)	0	0,78±0,19^{aB}	1,84±0,49^{bB}	1,30±0,51^{abB}	0,83±0,16^{aB}	0,81±0,06^{aC}
	7	0,56±0,15^{abAB}	1,21±0,21^{bcAB}	1,55±0,46^{cC}	0,40±0,15^{aB}	0,53±0,14^{abAB}
	14	0,43±0,09^{aA}	1,00±0,16^{bA}	1,19±0,25^{bB}	0,58±0,06^{aAB}	0,62±0,05^{aBC}
	21	0,37±0,08^{aA}	1,32±0,04^{bAB}	1,24±0,07^{bA}	0,35±0,03^{aA}	0,33±0,03^{aA}
Cohesividad (N)	0	-0,02±0,01^{b}	-0,03±0,01^{B}	-0,03±0,03^{B}	-0,04±0,02^{A}	-0,03±0,01^{A}
	7	-0,02±0,02^{b}	-0.05±0,00^{aA}	-0,06±0,00^{aA}	-0,02±0,01^{bB}	-0,03±0,00^{bB}
	14	-0,02±0,02^{c}	-0,04±0,01^{bAB}	-0,06±0,03^{aA}	-0,02±0,00^{cB}	-0,02±0,01^{cB}
	21	-0,02±0,01^{b}	-0,05±0,01^{aA}	-0,05±0,01^{aA}	-0,02±0,01^{bB}	-0,02±0,01^{bC}
Índice de viscosidad (N.s)	0	0,85±0,05^{dC}	-0,17±0,05^{aA}	-0,15±0,03^{aA}	-0,05±0,02^{bA}	-0,01±0,01^{bA}
	7	0,51±0,01^{cB}	-0,05±0,01^{abB}	-0,06±0,02^{aB}	-0,02±0,03^{bB}	-0,02±0,02^{bB}
	14	0,51±0,02^{bB}	-0,03±0,01^{aB}	-0,03±0,01^{aB}	-0,02±0,01^{aB}	-0,03±0,01^{aB}
	21	0,04±0,01^{bA}	-0,07±0,01^{aB}	-0,04±0,01^{bB}	-0,02±0,01^{bB}	-0,03±0,00^{bB}

$^{a-d}$ Las diferentes letras minúsculas en la misma fila indican diferencias significativas ($p < 0,05$) debido a la adición de pasta y harina de dátil. $^{A-C}$ Las diferentes letras mayúsculas en la misma columna indican diferencias significativas ($p > 0,05$) debido al tiempo de almacenamiento. ND: no detectado. Y3HD: yogur con 3% de harina de dátil; Y6HD: yogur con 6% de harina de dátil; Y3PD: yogur con 3% de pasta de dátil; Y6PD: yogur con 6% de pasta de dátil.

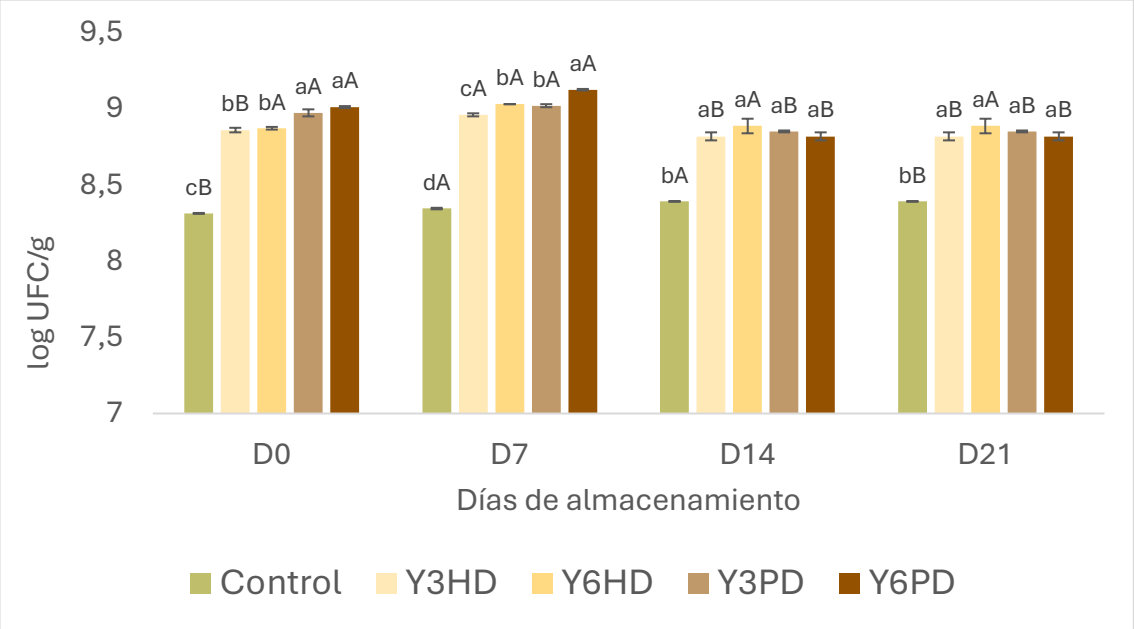


Figura 21. Recuento de *Lactobacillus* spp. en yogures enriquecidos con PAIs de dátiles durante 21 días de almacenamiento refrigerado.
Para el mismo tiempo, las diferentes letras minúsculas [a-c] indican diferencias significativas ($p < 0,05$) entre las formulaciones de yogur. Para la misma formulación, las diferentes letras mayúsculas [A-E] indican diferencias significativas ($p < 0,05$) entre los tiempos de almacenamiento.

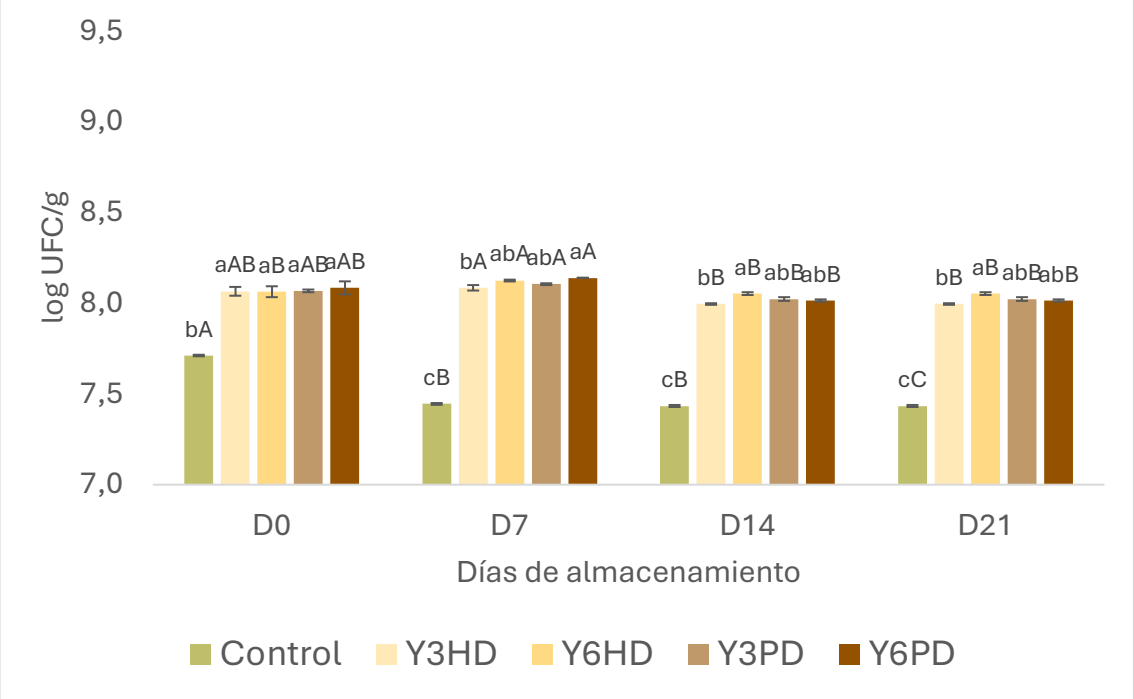


Figura 22. Recuento de *Streptococcus* spp. en yogures enriquecidos con PAIs de dátiles durante 21 días de almacenamiento refrigerado.
Para el mismo tiempo, las diferentes letras minúsculas [a-c] indican diferencias significativas ($p < 0,05$) entre las formulaciones de yogur. Para la misma formulación, las diferentes letras mayúsculas [A-E] indican diferencias significativas ($p < 0,05$) entre los tiempos de almacenamiento.

La adición de estos PAI mejoró el crecimiento y la estabilidad del cultivo iniciador de los yogures, lo que podría aumentar su capacidad probiótica. Por otro lado, la pequeña disminución del número de células viables de bacterias lácticas durante el almacenamiento se debe probablemente al aumento de la acidez y a la reducción del pH del yogur durante el almacenamiento refrigerado, lo que concuerda con lo reportado por otros estudios (Jaster et al., 2018; Mohamed-Ahmed et al., 2021; Du et al., 2023).

De los siete atributos evaluados durante el análisis sensorial del yogur (color, sabor, olor, dulzor, acidez, granulosidad y firmeza), solo mostraron diferencias significativas el color, el sabor y la granulosidad (figura 23). Cabe destacar que, a pesar de que la acidez aumentó (tabla 22), esta no supuso un impacto en cuanto a la percepción sensorial de la misma en el yogur. La adición de harina de dátil (a ambas concentraciones) dio lugar a unos yogures con una tonalidad más marrón y una textura más granulada, lo que redujo la puntuación de ambos atributos, dando lugar a una valoración negativa por parte de los panelistas. La mayor granulosidad detectada en los yogures con harina de dátil se debe a la insolubilidad de la misma, lo que concuerda con los resultados de otros estudios sobre yogures con adición de extractos vegetales ricos en fibra dietética (Sendra et al., 2008; Darwish et al., 2018; Jrad et al., 2022).

La adición de un 6% de harina de dátiles disminuyó ($p < 0,05$) la puntuación del sabor. Según los comentarios recibidos, los consumidores prefirieron los yogures control y los yogures con pasta de dátil añadida (3% y 6 %) a los yogures con harina de dátiles (3% y 6%). Sin embargo, se puede concluir que los yogures enriquecidos con PAI del dátil fueron bien aceptados de forma general, si se compara con la baja aceptación de otros yogures enriquecidos con extractos vegetales, tal y como se encuentra en la bibliografía (Hashim et al., 2009; Tseng y Zhao, 2013; Bertolino et al., 2015).

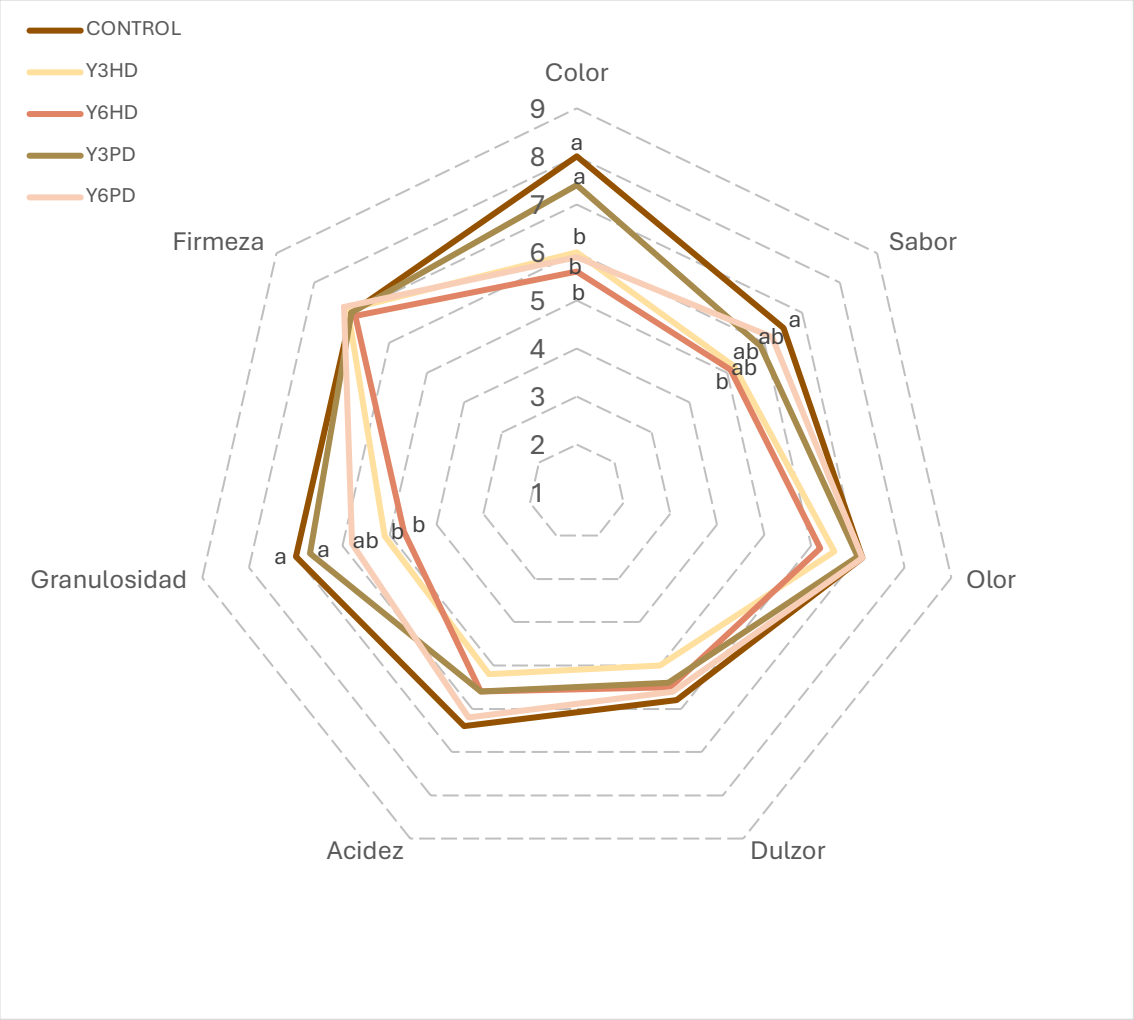


Figura 23. Evaluación sensorial de los yogures fortificados con pasta y harina de dátil Confitera.
[a-b]Diferentes letras muestran diferencias significativas entre muestras para un mismo parámetro.

4.4.2. Queso fresco elaborado con leche de cabra y fortificado con pasta de dátil confitera

Como del estudio anterior se dedujo que la pasta de dátil se comportaba de una mejor manera en la matriz láctea que la harina de dátil, se procedió a seleccionarla como el PAI más idóneo para la elaboración del queso fresco. La incorporación de pasta de dátil (4% y 8%) al queso fresco de leche de cabra no alteró el proceso de cuajado y desuerado del mismo, resultando un proceso tecnológicamente viable (figura 24).



Figura 24. Elaboración de los quesos frescos de cabra fortificados con pasta de dátil Confitera.

En la tabla 25 se muestran los resultados obtenidos tras la caracterización proximal del queso fresco, tanto de la muestra control como las dos muestras con adición de pasta de dátil. Como se puede observar, los quesos fortificados con pasta de dátil mostraron menor contenido en proteínas y mayor porcentaje de humedad ($p < 0,05$) que los controles, en cambio, no mostraron diferencias significativas en cuanto al contenido en grasa y cenizas ($p > 0,05$).

En referencia a estos resultados, es probable que ese aumento de humedad esté estrechamente relacionado con la capacidad de retención de agua que mostró la pasta de dátil. Además, hay que tener en cuenta que la composición química del queso depende de factores como la raza de la cabra, la dieta, la estación del año, su procesamiento, etc. Sin embargo, tanto en la leche como en los quesos, la grasa es el componente principal después del contenido en humedad, por ello, estos quesos mostraron valores dentro del rango normal para los quesos frescos elaborados con leche de cabra (Masotti et al., 2012; Kawęcka y Pasternak, 2022).

Tabla 25. Composición proximal de los quesos frescos de cabra enriquecidos con pasta de dátil Confitera.

	Proteínas (%)	Grasa (%)	Cenizas (%)	Humedad (%)
Control	13,52±0,16^{a}	22,87±1,50^{a}	2,33±0,06^{a}	60,23±0,66^{b}
DP4	12,42±0,25^{b}	22,29±0,57^{a}	2,19±0,10^{a}	63,07±0,43^{a}
DP8	12,65±0,42^{b}	22,23±0,72^{a}	2,13±0,15^{a}	62,94±0,60^{a}

$^{a-b}$Diferentes letras en la misma columna muestran diferencias significativas entre muestras para un mismo parámetro.

A continuación, en la tabla 26 se muestran las propiedades fisicoquímicas del queso control y de los quesos enriquecidos con pasta de dátil al 4% y 8%. La actividad del agua (Aw) es un factor biofísico importante en la elaboración del queso, ya que implica la transformación de la leche, un líquido perecedero, en un producto semisólido con una vida útil limitada, dependiendo del tipo de queso. Se ha reportado que son la sal añadida y el proceso de escurrido, los factores que más

influyen en la actividad de agua de los quesos. No hubo diferencias significativas de Aw en los quesos elaborados, ($p > 0,05$), encontrándose dentro del rango normal para los quesos frescos (Trmčić et al., 2017). También el pH mostró unos valores dentro del rango habitual de los quesos. A pesar de esto, se observó que la adición de pasta disminuyó ligeramente los valores obtenidos, siendo similar a otros estudios de quesos con extractos de frutas ricos en compuestos fenólicos (Jeong et al., 2017; Soliman et al., 2022; Ferreira et al., 2024). De acuerdo con algunos autores (Nontasan et al., 2012; Lipșa et al., 2024), los cambios de color en los productos lácteos es un factor importante estrechamente relacionado con la calidad e idoneidad de los alimentos. En la tabla 26, se pueden observar las propiedades colorimétricas de los quesos elaborados, donde tanto la coordenada b* (amarillo/azul) como el parámetro C* (saturación) no mostraron diferencias significativas ($p > 0,05$), por lo tanto, no se vieron afectadas por la adición de pasta de dátil. En cuanto a la luminosidad (L*) y al tono (h*) se observó una reducción de los valores obtenidos, a su vez, relacionada con el aumento del porcentaje de adición de pasta de dátil ($p < 0,05$). Además, varios autores han informado de esta disminución debido a la adición de extractos vegetales (Giroux et al., 2013; Caleja et al., 2015) y de una mayor luminosidad (L*) en quesos elaborados con leche de cabra en lugar de vaca u oveja (Milovanovic et al., 2020). Por otro lado, los quesos con pasta de dátil adicionada mostraron valores de la coordenada a* más altos que los de control ($p < 0,05$). Esta disminución de los valores L* y h* y el aumento de los valores a* en los quesos con incorporación de pasta de dátil podría atribuirse a las propiedades cromáticas de pasta (tabla 13). La blancura (IB) es uno de los parámetros más importantes en los quesos frescos, los cuales suelen conservar el blanco característico de la leche debido a una conversión del β-caroteno (amarillo anaranjado) en vitamina A (incolora) (Milovanovic et al., 2020). La adición de pasta de dátil disminuyó el índice de blancura en los quesos, aunque dicha variación solo fue significativa cuando se añadió un 8 % de pasta de dátil ($p < 0,05$). Estos cambios de color en los quesos también se reflejan en las diferencias de color (ΔE*) entre los quesos con PD adicionada y los quesos control. El queso DP4 presentó diferencias

de color respecto al control por debajo de 3 unidades, lo que generalmente se considera imperceptible para el ojo humano (Martínez et al., 2001).

Tabla 26. Propiedades fisicoquímicas de los quesos frescos de cabra enriquecidos con pasta de dátil Confitera.

	Control	DP4	DP8
Aw	0,96±0,00^{a}	0,96±0,01^{a}	0,96±0,00^{a}
pH	5,73±0,04^{a}	5,43±0,02^{c}	5,57±0,03^{b}
L*(D65)	82,72±0,72^{a}	79,90±3,06^{ab}	78,02±3,73^{b}
a*(D65)	-1,50±0,05^{b}	-0,57±0,70^{ab}	0,02±1,00^{a}
b*(D65)	6,63±0,18^{a}	7,64±1,31^{a}	8,82±3,16^{a}
C*(D65)	6,80±0,18^{a}	7,69±1,29^{a}	8,85±3,20^{a}
h(D65)	102,78±0,58^{a}	94,91±5,05^{ab}	90,90±4,99^{b}
IB	81,42±0,70^{a}	80,31±0,55^{a}	76,74±1,54^{b}
ΔE*	-	1,33±0,52^{b}	5,29±1,35^{a}

$^{a-c}$Diferentes letras en la misma fila muestran diferencias significativas entre muestras para un mismo parámetro.

Los parámetros relacionados con la textura de los quesos elaborados con pasta de dátil, firmeza, adhesividad, elasticidad, cohesividad y resiliencia se muestran en la tabla 27. Como puede observarse en dicha tabla, todos los parámetros de textura fueron afectados por la adición de pasta de dátil, excepto la cohesividad y la resiliencia, las cuales no mostraron diferencias significativas ($p > 0,05$). Todos los quesos mostraron valores de cohesividad elevados (> 0,85), ya que valores cercanos a 1,0 indican que el queso puede soportar un ciclo de compresión sin desintegrarse, en cambio, valores cercanos a 0 hacen referencia a una desintegración completa (Paredes et al., 2022). La resiliencia mide la capacidad del queso para recuperar su forma después del primer ciclo de compresión, la cual no se vio afectada. La firmeza mostró un cambio más notable con las diferentes concentraciones de pasta de dátil: disminuyó con la adición de concentraciones bajas (DP4), pero aumentó con concentraciones más altas (DP8), lo que podría indicar que la adición afectó al proceso de elaboración del queso. Los cambios de pH en el queso al que se le añadió pasta de dátil (tabla 26) y su interacción con las enzimas (cuajo) y los cultivos iniciadores son cruciales para la retención de humedad y la formación de la cuajada (descomposición de las proteínas de la leche). Estos factores influyen en la firmeza, la elasticidad y la estructura de la cuajada, lo que en última instancia afecta a la textura final del queso (Milovanovic et al., 2020). Por otro lado, la elasticidad, relacionada con la recuperación del

material y sus propiedades viscoelásticas (Johnson, 2023), disminuyó cuando se incorporó pasta de dátil al 4 % ($p < 0,05$), en cambio, mostró valores similares en los quesos control y DP8 ($p > 0,05$). Por el contrario, la adhesividad, la cual mide el esfuerzo necesario para superar las fuerzas de atracción entre la superficie del queso y las superficies con las que entra en contacto (lengua, dientes o paladar), solo se vio afectada con la adición del 8 %. Esto puede estar relacionado con el elevado contenido en azúcares de la pasta de dátil (tabla 12) la cual puede aumentar significativamente la adhesividad de los quesos.

Tabla 27. Propiedades texturales de los quesos frescos de cabra enriquecidos con pasta de dátil Confitera.

	Firmeza (N)	Adhesividad (N*s)	Elasticidad	Cohesividad	Resiliencia
Control	2,23±0,40^{b}	-0,01±0,00^{a}	0,18±0,05^{ab}	0,91±0,05^{a}	0,50±0,02^{a}
DP4	0,74±0,13^{c}	-0,01±0,01^{a}	0,12±0,03^{c}	0,85±0,08^{a}	0,45±0,10^{a}
DP8	3,16±0,23^{a}	-0,04±0,01^{b}	0,22±0,03^{a}	0,85±0,06^{a}	0,41±0,03^{a}

$^{a-c}$Diferentes letras en la misma columna muestran diferencias significativas entre muestras para un mismo parámetro.

En la tabla 28 se muestra el perfil de azúcares y ácidos orgánicos de los quesos con pasta de dátil. En cuando a los azúcares detectados, el mayoritario fue la lactosa (control: 27,35 mg/g; DP4: 27,33 mg/g; DP8: 27,97 mg/g), el cual no mostró diferencias significativas ($p > 0,05$) entre la muestra control y las muestras con pasta de dátil. En cuanto a la fructosa, no fue detectada en la muestra control, mientras que sí se detectó en DP4 y DP (1,81 mg/g y 3,66 mg/g, respectivamente). Como puede observarse, el contenido en fructosa incrementó conforme aumentaba el contenido en pasta de dátil, esto es debido a la cantidad de fructosa que contiene el dátil (tabla 12).

Tabla 28. Contenido de azúcares (lactosa y fructosa) y ácidos orgánicos (ácido cítrico y láctico) en los quesos frescos enriquecidos con pasta de dátil Confitera.

	Lactosa	Fructosa	Ácido cítrico	Ácido láctico
Control	27,35±0,14^{a}	ND	3,64±0,07^{a}	8,64±0,19^{b}
DP4	27,33±0,24^{a}	1,81±0,02^{b}	3,56±0,10^{ab}	12,87±0,02^{a}
DP8	27,97±0,13^{a}	3,66 ±0,04^{a}	3,31±0,01^{b}	14,50±1,36^{a}

$^{a-b}$Diferentes letras en la misma columna muestran diferencias significativas entre muestras para un mismo parámetro. ND: no detectado.

Como era de esperar, en referencia a los ácidos orgánicos, el mayoritario fue el ácido láctico, seguido del ácido cítrico. En este caso ocurre algo similar a la

fructosa, el ácido láctico aumentó con la incorporación gradual de pasta de dátil (control: 8,64 mg/g; DP4: 12,87 mg/g; DP8: 14,50 mg/g) que a su vez está relacionado con el recuento de bacterias acidolácticas (figura 25), las cuales aumentaron con la adición de pasta de dátil con respecto al control (*Lactobacillus* spp: 7,43 a 8,30 $\log_{10}$ UFC/g; *Streptococcus* spp: 6,11 a 7,45 $\log_{10}$ UFC/g). En cambio, al contrario que en el resto de compuestos, el ácido cítrico disminuyó ligeramente con la presencia de pasta de dátil (3,64 a 3,31 mg/g), siendo proporcional al aumento de dátiles en las muestras. Algunos autores mostraron un perfil de azúcares y ácidos orgánicos similar en el queso de cabra a día 1 de maduración (Moreira et al., 2020), por lo que se podría concluir que estos valores se encuentran dentro de los rangos establecidos para este tipo de queso.

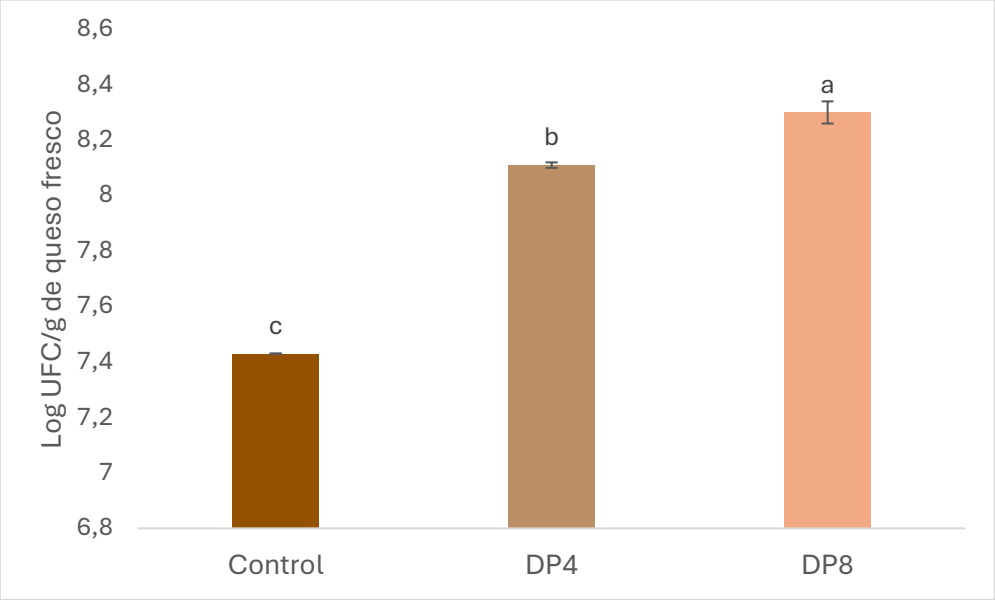


Figura 25. Recuento de *Lactobacillus spp*. presentes en los quesos frescos de cabra enriquecidos con pasta de dátil Confitera.
[a-c]Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

En la siguiente tabla (tabla 29) se muestra el contenido en minerales de los quesos fortificados con pasta de dátil. A simple vista se puede observar que todos los minerales analizados (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn) en los quesos fueron aumentando con el incremento de la pasta de dátil incorporada. Los minerales con un contenido más destacable fueron el Ca, el K, el Mg, Na, y el P. De todos ellos, el potasio fue el que más aumentó con respecto al queso control (44%) ya que como

se mostró anteriormente (tabla 12) la pasta de dátil es una fuente excelente de potasio (658 mg/100g). Existen numerosos factores que pueden influir en el contenido de minerales de los quesos de cabra, lo que da lugar a una gran variabilidad en los datos comunicados. Sin embargo, nuestros resultados se encuentran dentro de los rangos reportados por otros autores (Moreno-Rojas et al., 2010; Herman-Lara et al., 2019)

Tabla 29. Contenido de minerales en los quesos frescos enriquecidos con pasta de dátil Confitera.

	Control	DP4	DP8
Ca	503,97±2,94^{c}	517,82±6,43^{b}	536,86±4,51^{a}
Cu	0,01±0,00^{c}	0,03±0,01^{b}	0,06±0,01^{a}
Fe	0,41±0,01^{b}	0,55±0,04^{a}	0,63±0,05^{a}
K	98,81±5,06^{b}	105,87±1,51^{b}	141,00±3,90^{a}
Mg	17,68±0,11^{b}	17,71±0,07^{b}	22,37±0,56^{a}
Mn	0,07±0,00^{b}	0,09±0,01^{b}	0,12±0,00^{a}
Na	636,49±14,25^{b}	669,96±17,84^{b}	778,76±6,63^{a}
P	260,25±3,80^{a}	257,22±1,06^{a}	263,49±5,33^{a}
Zn	1,43±0,04^{b}	1,46±0,04^{b}	1,98±0,14^{a}

$^{a-c}$Diferentes letras en la misma fila muestran diferencias significativas entre muestras para un mismo parámetro.

En la figura 26 se muestra la microestructura de los quesos elaborados mediante microscopía confocal de láser de barrido, la cual se utiliza para estudiar sistemas de gel de proteínas alimentarias. En estas imágenes se puede observar cómo afectó a la microestructura del queso fresco la adición de pasta de dátil. En la figura se muestran los glóbulos de grasa (rojo) y los espacios porosos (negro) dispersos por toda la matriz proteica (verde). En el queso control, los glóbulos de grasa se dispersaban aleatoriamente dentro de la matriz de caseína, mostrando una forma esférica regular sin presencia de polisacáridos. Sin embargo, en las muestras DP4 y DP88 se observaron fibras de pasta de dátil, considerablemente más grandes que los glóbulos de grasa (50 µm frente a 3-4 µm).

Como se muestra en la figura, la estructura de la red proteica no difería con respecto al queso control por lo que la adición de pasta de dátil no alteró la microestructura del queso. Debido a esto, se puede decir que la pasta de dátil se integró en la matriz proteica, conservando la red proteico-lipídica característica del queso fresco de cabra. Además, las imágenes ampliadas de los quesos con pasta de dátil mostraron glóbulos de grasa y proteínas incrustados en las fibras de dátil, lo que confirma su incorporación satisfactoria al queso fresco de cabra. Este

estudio mostró una red más compacta y con menos poros que las reportadas por otros autores (Wang et al., 2023b).

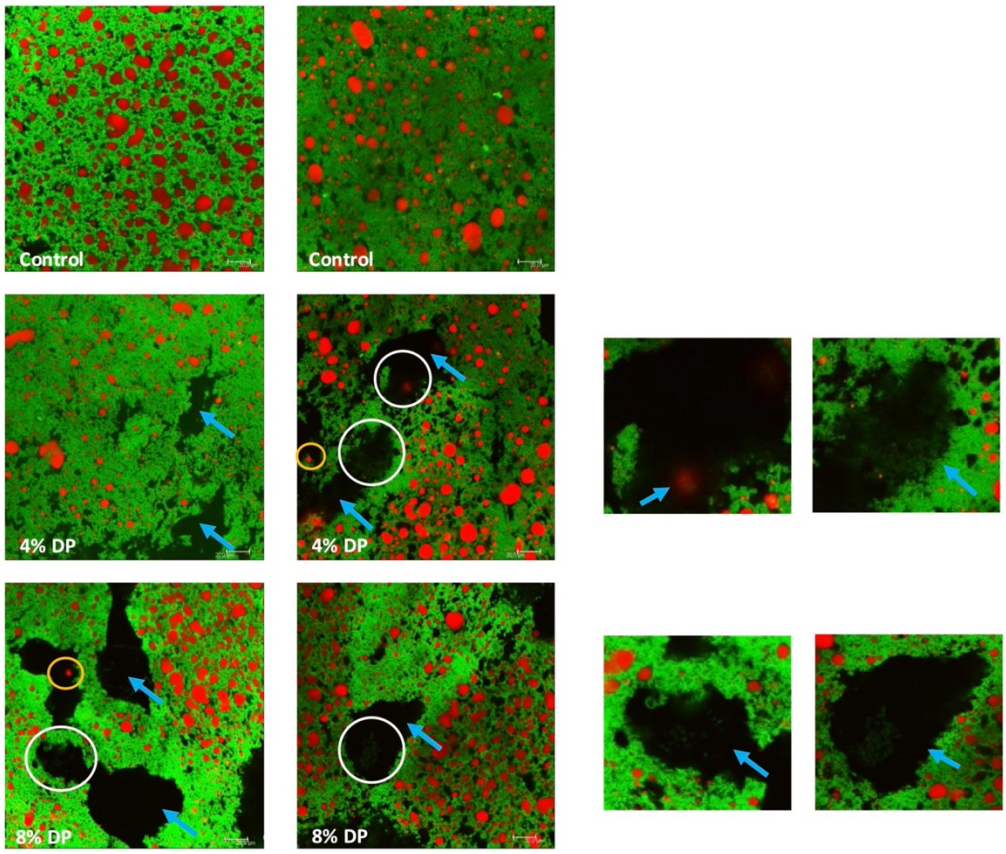


Figura 26. Efecto de la incorporación de pasta de dátil Confitera en la microestructura del queso fresco de cabra.
Las flechas azules señalan las fibras de dátil dentro de los glóbulos de grasa y proteínas. Las proteínas teñidas con Fast Green aparece en verde y los cuerpos lipídicos teñidos con rojo Nilo aparecen en rojo. Barras de escala: 20 µm.

En la evaluación sensorial de los quesos frescos elaborados con leche de cabra y enriquecidos con pasta de dátil, se analizaron las propiedades de color, olor, sabor, dulzor, salado, firmeza, granulosidad, fracturabilidad y aceptabilidad general (figura 27 y 28). En la Figura 27 se presentan los ocho atributos sensoriales evaluados. De ellos, únicamente el color y la granulosidad mostraron diferencias significativas ($p < 0,05$). En comparación con el queso control, los quesos con dátil obtuvieron puntuaciones más bajas en color, firmeza y granulosidad; sin embargo, en todos los casos las valoraciones fueron superiores a 5 en la escala utilizada. Cabe destacar que las diferencias en color y firmeza percibidas tras el estudio sensorial, coinciden con los resultados previamente reportados en las tablas anteriores (tabla 26 y 27). La adición de pasta de dátil no afectó a los atributos

referentes al aroma, el sabor, el dulzor, el sabor salado y la fracturabilidad de los quesos evaluados. Además, es importante señalar que la incorporación de pasta de dátil, a pesar de su elevado contenido en fructosa y ácido láctico, no afectó negativamente a la percepción del dulzor de los quesos, obteniendo valores similares a los obtenidos en los quesos control.

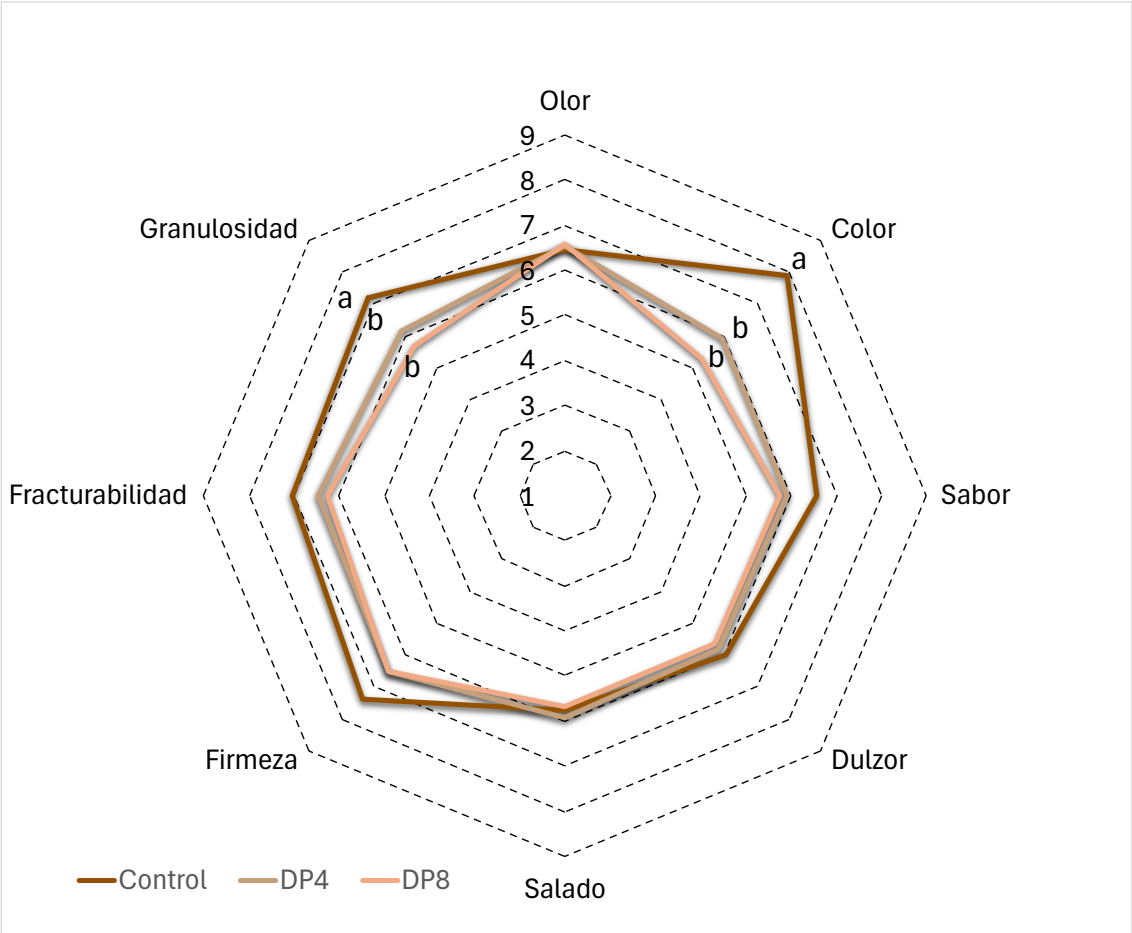


Figura 27. Evaluación sensorial de los quesos frescos de cabra enriquecidos con pasta de dátil Confitera.
[a-b]Diferentes letras muestran diferencias significativas entre muestras para un mismo parámetro.

Los quesos de las muestras control presentaron una puntuación significativamente superior ($p < 0,05$) en términos de aceptabilidad general, en comparación con aquellos elaborados con pasta de dátiles, sin que se observaran diferencias destacables entre las dos concentraciones de pasta de dátil adicionadas. No obstante, todas las puntuaciones se mantuvieron por encima de los 5 puntos, lo que indica una aceptación globalmente positiva de todos los

quesos frescos de cabra. Dado que la incorporación de dátiles o sus derivados no constituye una práctica habitual en la industria láctea, la realización de estudios que avalen su viabilidad tecnológica permitiría diversificar la oferta de productos lácteos enriquecidos, contribuyendo al desarrollo del sector y ampliando las opciones disponibles para los consumidores. Desde la perspectiva científico-tecnológica, los resultados obtenidos demuestran que la incorporación de pasta de dátil en concentraciones de hasta un 8 % no interfiere en el proceso de elaboración del queso fresco de cabra, lo que indica que no sería necesaria la implementación de procesos o equipamiento adicional al utilizado tradicionalmente.

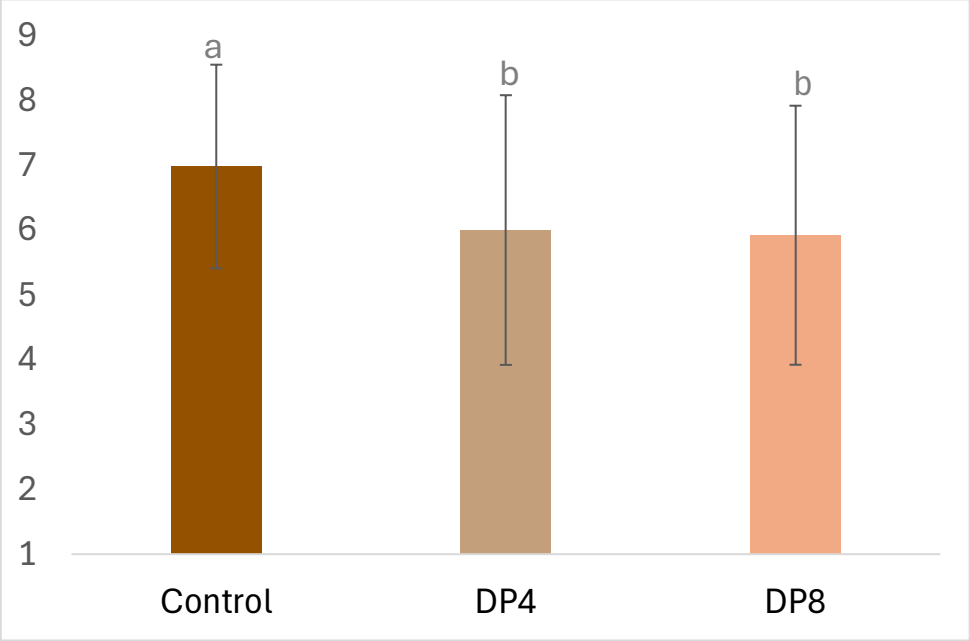


Figura 28. Aceptación general de los quesos frescos de cabra enriquecidos con pasta de dátil.
[a-b]Diferentes letras muestran diferencias significativas entre muestras para un mismo parámetro.

4.4.3. Kéfir elaborado con leche de cabra y fortificado con pasta de dátil Confitera y suero de queso fresco de cabra con pasta de dátil Confitera

De la elaboración del queso fresco de leche de cabra con pasta de dátil del trabajo anterior, se obtuvo como coproducto el suero de queso, que se usó como ingrediente en la elaboración de este kéfir. La fortificación del kéfir con pasta de dátil (3% y 6%) resultó un proceso tecnológicamente viable. Sin embargo, la aplicación del suero lácteo - el cual se obtuvo durante la elaboración de queso

fresco con un 8% de pasta de dátil - como sustituto (25% y 50%) de la leche de cabra, resultó inviable tecnológicamente por una separación muy evidente de las fases grasas y acuosas de ambos ingredientes.

En la tabla 30 se muestran los valores obtenidos tras el estudio de la composición proximal de los diferentes kéfires elaborados. Tanto en el porcentaje de cenizas como de humedad se observó un aumento en todas las muestras elaboradas con respecto al control, siendo la muestra con mayor sustitución de leche por suero (WH50) la que obtuvo los valores mayores ($p < 0,05$). Este aumento de humedad en la muestra WH50 se puede atribuir al mismo suero, ya que su contenido en agua era superior que el de la leche (Sanmartín et al., 2012; Galdino et al., 2020). Por otro lado, el contenido en grasa y proteínas disminuyó significativamente ($p < 0,05$) tanto en las muestras con pasta de dátil como con suero lácteo, decreciendo del 4,47% (control) al 2,68% (WH50) de grasa y del 3,73% (control) al 1,63% (WH50) de proteínas. Cabe destacar que todos los valores observados se encontraban dentro de los rangos de composición proximal para el kéfir de leche de cabra según varios autores (Gürsoy et al., 2020; Satir y Guzel-Seydim, 2023; Wang et al., 2023a). En estudios similares, también se reportaron niveles más bajos de proteínas y grasas y más altos de cenizas tras la incorporación de extractos vegetales en bebidas fermentadas de leche de cabra (Tawfek et al., 2021; Fiqri Erzhad et al., 2022).

Tabla 30. Composición proximal de los kéfires de cabra reformulados con pasta de dátil Confitera y con suero lácteo.

	Proteínas (%)	Grasa (%)	Cenizas (%)	Humedad (%)	SST (%)
Control	3,73±0,12^{a}	4,87±0,04^{a}	0,72±0,01^{c}	85,42±0,32^{c}	4,85±0,21^{a}
DP3	2,39±0,01^{b}	3,04±0,00^{b}	0,76±0,02^{bc}	86,56±0,83^{bc}	4,36±0,06^{b}
DP6	2,46±0,03^{b}	3,22±0,80^{b}	0,85±0,05^{b}	86,73±0,19^{ab}	4,40±0,07^{b}
WH25	2,11±0,07^{c}	3,17±0,04^{b}	0,82±0,02^{bc}	86,08±0,59^{bc}	3,81±0,01^{c}
WH50	1,63±0,01^{d}	2,68±0,17^{c}	0,98±0,04^{a}	87,96±0,62^{a}	3,16±0,05^{d}

$^{a-d}$ Las diferentes letras entre filas indican diferencias significativas ($p < 0,05$). DP3: kéfir con 3% de pasta de dátil; DP6: kéfir con 6% de pasta de dátil; WH25: kéfir con 25% de leche sustituida por suero de queso con pasta de dátil; WH50: kéfir con 50% de leche sustituida por suero de queso con pasta de dátil.

Por otro lado, en la tabla 31 se muestran las propiedades fisicoquímicas de los kéfires elaborados, las cuales son consideradas de las propiedades que más

influyen en su calidad, ya que están relacionadas con el proceso de fermentación láctica, la fluidez y el color (Saygili et al., 2022). Como se observa, todos los parámetros medidos mostraron diferencias significativas entre las muestras elaboradas ($p < 0,05$). La acidez y el pH tienen un papel muy importante en la textura y el sabor de los productos lácteos fermentados como el kéfir. El pH disminuyó ligeramente con la adición de pasta de dátil, además, se observó un comportamiento similar en las muestras con suero de queso, donde se obtuvieron valores parecidos a las muestras con pasta de dátil, encontrándose estos valores dentro del rango normal del kéfir (Simova et al., 2002; Chen et al., 2009). Es importante destacar que ninguno de los valores obtenidos se encontró por debajo de 4,0, ya que niveles inferiores se consideran perjudiciales para los probióticos (Taheur et al., 2023). De acuerdo con otros autores, el pH de los kéfires también disminuyó con la adición de extractos de frutas como cáscara de granada, de mango, higo chumbo, limón, etc. (Chan et al., 2018; Vicenssuto y de Castro, 2020; Taheur et al., 2023). Es importante destacar que los extractos de frutas pueden servir como fuente de azúcares naturales y polisacáridos, los cuales pueden convertirse en glucosa y transformarse en ácido láctico debido a la acción de microorganismos (Vicenssuto y de Castro, 2020). En referencia a la consistencia del kéfir, varios estudios han demostrado que el kéfir elaborado con leche de cabra y oveja tiene una viscosidad considerablemente menor en comparación con el elaborado con leche de vaca, y que el uso de granos de kéfir a altas temperaturas da lugar a valores de viscosidad más altos (Ari et al., 2012; Vianna et al., 2017; Saygili et al., 2022).

En cuanto a la adición de pasta de dátil, esta no modificó la viscosidad en las muestras de kéfir en ninguna de las concentraciones añadidas de pasta de dátil (3 % y 6 %) ($p < 0,05$). En cambio, se apreció una notable disminución de la viscosidad en las muestras con suero lácteo (WH25 y WH50), siendo uno de los factores que invalidó su aplicación tecnológica en la elaboración del kéfir. Como se ha observado en los estudios anteriores, la adición de pasta de dátil en productos lácteos como el yogur o el queso no provocó cambios significativos en su textura debido a una integración perfecta en la matriz de la leche.

Tabla 31. Propiedades fisicoquímicas de los kéfires de cabra fortificados con pasta de dátil Confitera.

	Control	DP3	DP6	WH25	WH50
pH	4,32±0,02^{a}	4,24±0,01^{b}	4,21±0,01^{c}	4,26±0,01^{b}	4,20±0,01^{c}
Acidez (ºD)	94,00±2,83^{a}	101,00±1,41^{a}	96,50±2,12^{a}	64,00±1,41^{b}	66,00±1,41^{c}
Viscosidad (mPa.s)	1125,80± 60,37^{a}	1193,10±1,18^{a}	1141,10±6,20^{a}	451,60±12,82^{b}	113,27±4,02^{b}
L*(D65)	81,186±0,74^{a}	73,83±0,71^{b}	77,36±0,57^{b}	78,48±0,57^{c}	61,12±0,39^{d}
a*(D65)	-0,74±0,09^{c}	0,77±0,18^{a}	0,67±0,08^{a}	-0,23±0,03^{b}	-0,62±0,05^{c}
b*(D65)	5,14±0,18^{b}	8,67±0,54^{a}	9,18±0,53^{a}	8,39±0,19^{a}	4,55±0,02^{b}
C*(D65)	5,19±0,17^{b}	8,70±0,54^{a}	9,21±0,53^{a}	8,39±0,19^{a}	4,59±0,03^{b}
h*(D65)	98,24±1,16^{a}	84,92±1,14^{c}	85,81±0,19^{c}	91,59±0,14^{b}	97,73±0,61^{a}
IB	80,48±0,72^{a}	75,73±0,41^{b}	76,60±0,72^{b}	72,51±0,62^{c}	60,84±0,38^{d}

$^{a-d}$ Las diferentes letras entre columnas indican diferencias significativas ($p < 0,05$). DP3: kéfir con 3% de pasta de dátil; DP6: kéfir con 6% de pasta de dátil; WH25: kéfir con 25% de leche sustituida por suero de queso con pasta de dátil; WH50: kéfir con 50% de leche sustituida por suero de queso con pasta de dátil.

El color de los alimentos, en este caso el color blanco del kéfir, desempeña un papel fundamental en cuanto a la aceptación por parte de los consumidores y en sus decisiones de compra. Hay que tener en cuenta que, en los productos lácteos, el color puede verse afectado por numerosos factores como el tipo de leche, el proceso de fermentación, ingredientes o aditivos añadidos, etc. En este estudio, todas las muestras de kéfires elaborados mostraron valores de L* más bajos con respecto al control, siendo WH50 el que obtuvo menor luminosidad ($p < 0,05$). Esta disminución de L* se debió a la adición de pasta de dátil, al igual que ocurre en otros estudios (Goncu et al., 2017; Taheur et al., 2023). En cuanto a los valores a* y b*, se observó un aumento significativo ($p < 0,05$) en las muestras de kéfir con adición de dátil (DP3 y DP6), sin diferencias entre las concentraciones en comparación con el kéfir de control. Estas muestras mostraron los valores a* y b* más altos que el resto ($p < 0,05$). Cabe destacar que estos cambios de color relacionados con las coordenadas a* y b* (rojo y amarillo) están relacionados con la adición de pasta de dátil, por su contenido en pigmentos naranja-amarillo-rojo (carotenos y antocianinas) y por los compuestos generados durante las reacciones de Maillard (Al-Qarni y Bazzi, 2020). La adición de pasta de dátil aumentó los valores de C*, mientras que el uso de suero de queso al 50 % dio lugar a valores de saturación similares a los del control ($p > 0,05$). El tono del color disminuyó en el kéfir reformulado excepto en WH50, que mostró un tono similar al del control ($p < 0,05$). La reformulación del kéfir provocó un cambio en el tono, pasando de un tono amarillo limón (control) a amarillo anaranjado (DP3 y DP6) (IRANOR, 1981; Gürsoy et al., 2020). En cambio, el índice de blancura disminuyó con la adición de la pasta de dátil, no mostrando diferencias entre las concentraciones de pasta de dátil adicionada ($p > 0,05$). El kéfir con suero de queso en la concentración más alta (WH50) mostró el IB más bajo ($p < 0,05$). Sin embargo, los valores de IB obtenidos para todas las muestras de kéfir reformulado se encontraban dentro del rango de los valores reportados para las muestras de kéfir comercial (Gürsoy et al., 2020).

En la siguiente tabla 32 se muestra el perfil de minerales de las muestras elaboradas. De todos los minerales detectados destacaron principalmente Na, P,

K, Mg y Ca. Los aumentos observados en el contenido de Ca, K y Mg en el kéfir enriquecido con pasta de dátil se debieron principalmente al contenido mineral de la propia pasta de dátiles (tabla 12). La adición de pasta de dátil dio lugar a un aumento significativo de los niveles de Ca, P y Zn, mientras que las muestras que contenían suero de queso mostraron los valores más bajos de estos minerales ($p < 0,05$). Por otro lado, el contenido de hierro disminuyó con la adición excepto en WH25, la cual mantuvo el mismo valor que en el kéfir de control. Sin embargo, estas diferencias fueron prácticamente insignificantes, ya que las concentraciones de Fe en todas las muestras fueron extremadamente bajas (0,04-0,05 mg/100 g). En cuanto al Na, fue el mineral más destacado, aumentando significativamente en el kéfir formulado con suero de queso, obteniendo valores casi 5 veces superiores (WH50) en comparación con el control (95,06 frente a 423,58 mg/100 g). Esto se debe a que se encuentra presente en concentraciones mayores en el suero de queso, además, su contenido puede verse influido por la cantidad de sal empleada durante la elaboración del queso. Este factor podría explicar el incremento de sodio observado en el kéfir formulado con suero de queso en comparación con el producto control o con aquellos que incorporan pasta de dátiles. Por el contrario, elementos como cobre, hierro y zinc, los cuales presentan una mayor afinidad por las caseínas en la leche de rumiantes, se detectan en concentraciones más bajas en el suero (Sousa et al., 2019).

Tabla 32. Contenido de minerales (mg/100 g) de los kéfires de cabra fortificados con pasta de dátil Confitera.

	Control	DP3	DP6	WH25	WH50
Ca	82,09±1,70^{b}	88,16±0,86^{a}	88,68±1,29^{a}	80,20±0,28^{b}	64,04±1,28^{c}
Cu	0,01±0,00^{b}	0,01±0,00^{ab}	0,02±0,00^{a}	0,01±0,00^{b}	0,01±0,00^{b}
Fe	0,05±0,00^{a}	0,04±0,00^{b}	0,04±0,00^{b}	0,05±0,00^{a}	0,04±0,00^{b}
K	96,64±1,84^{b}	131,25±0,45^{a}	131,37±0,75^{a}	97,74±1,81^{b}	95,45±2,48^{b}
Mg	8,47±0,11^{c}	10,39±0,10^{b}	11,43±0,08^{a}	8,27±0,45^{c}	8,04±0,19^{c}
Mn	0,01±0,00^{b}	0,01±0,00^{b}	0,02±0,00^{a}	0,01±0,00^{b}	0,01±0,00^{b}
Na	95,06±5,25^{d}	104,83±1,57^{c}	111,23±3,37^{c}	376,86±0,84^{b}	423,58±0,66^{a}
P	54,33±0,01^{c}	58,06±0,77^{b}	64,61±0,83^{a}	55,25±0,68^{c}	39,92±0,63^{d}
Zn	0,23±0,01^{c}	0,30±0,001^{b}	0,36±0,02^{a}	0,22±0,01^{c}	0,17±0,01^{d}

$^{a-d}$ Las diferentes letras entre columnas indican diferencias significativas ($p < 0,05$). DP3: kéfir con 3% de pasta de dátil; DP6: kéfir con 6% de pasta de dátil; WH25: kéfir con 25% de leche sustituida por suero de queso con pasta de dátil; WH50: kéfir con 50% de leche sustituida por suero de queso con pasta de dátil.

Los resultados obtenidos de azúcares y ácidos orgánicos se muestran en la tabla 33. Estos resultados mostraron que el ácido láctico es el único detectado en todas las formulaciones. Los niveles más bajos (3,21 mg/g y 2,89 mg/g) se observaron en las muestras que contenían suero, este contenido reducido de ácido láctico en el suero es el resultado del proceso de elaboración del queso, que incluye la pasteurización de la leche y la adición de cuajo para coagular la caseína y formar cuajada, sin una actividad significativa de los lactobacilos para convertir la lactosa en ácido láctico (Borba et al., 2022). Mientras, en las muestras con pasta de dátil (DP3 y DP4) se observó un aumento del ácido láctico con respecto al control, debido principalmente al hecho de que las bacterias lácticas, como *Lactobacillus* spp., utilizan la glucosa como sustrato durante la fermentación para producir ácido láctico (Wang et al., 2021). Para los azúcares, la lactosa fue el azúcar predominante mientras que la fructosa solo se detectó en DP3 y DP4, debido a su presencia en la pasta de dátil (137,72 mg/g) (tabla 12). En cambio, los niveles más altos de lactosa se observaron en las muestras enriquecidas con suero de queso, debido principalmente al alto contenido natural de lactosa en el suero (Sousa et al., 2019; Borba et al., 2022). Sin embargo, la adición de pasta de dátil no afectó al contenido de lactosa del kéfir, cuyos valores se mantuvieron comparables a los del control ($p < 0,05$).

Tabla 33. Contenido de azúcares (lactosa y fructosa) y de ácidos orgánicos (ácido láctico) de los kéfires de cabra fortificados con pasta de dátil Confitera.

	Ácido láctico	Fructosa	Lactosa
Control	5,55±0,16^{b}	ND	27,06±0,89^{c}
DP3	6,28±0,07^{a}	4,37±0,02^{b}	27,25±0,72^{c}
DP6	6,42±0,09^{a}	5,68±0,02^{a}	26,93±0,19^{c}
WH25	3,21±0,18^{c}	ND	34,22±0,04^{b}
WH50	2,89±0,17^{d}	ND	36,38±0,03^{a}

$^{a-d}$ Las diferentes letras entre filas indican diferencias significativas ($p < 0,05$). DP3: kéfir con 3% de pasta de dátil; DP6: kéfir con 6% de pasta de dátil; WH25: kéfir con 25% de leche sustituida por suero de queso con pasta de dátil; WH50: kéfir con 50% de leche sustituida por suero de queso con pasta de dátil.

Las bacterias lácticas del kéfir cumplieron con el mínimo recomendado de 106 UFC/mL para las bacterias probióticas vivas en los productos alimenticios probióticos (figura 29) (Marinova et al., 2019). En cuanto al recuento de *Lactobacillus* spp., se observó un aumento significativo en la incorporación de

pasta de dátil y suero de queso (enriquecido con un 8 % de pasta de dátil), con valores que oscilaron entre 8,21 y 8,47 log UFC/ml. Es bastante destacable que este aumento significativo en las formulaciones enriquecidas con pasta de dátil y suero lácteo sugiere que su potencial probiótico podría estar relacionado con la actividad prebiótica previa de pasta de dátil, como se mostró en los estudios preliminares. Además, la mejora del crecimiento microbiano en las muestras enriquecidas con pasta de dátil está relacionado con su contenido en minerales y compuestos fenólicos, los cuales servirían como sustratos prebióticos en productos fermentados funcionales. Sin embargo, la interacción entre las bacterias y los compuestos fenólicos es compleja y puede verse influida por factores como el tipo y la concentración del sustrato y la cepa bacteriana específica implicada (de Oliveira et al., 2020; Wang et al., 2022). También destaca su contenido en hidratos de carbono, el cual estimularía el crecimiento de las bacterias lácticas (Oliveira et al., 2022).

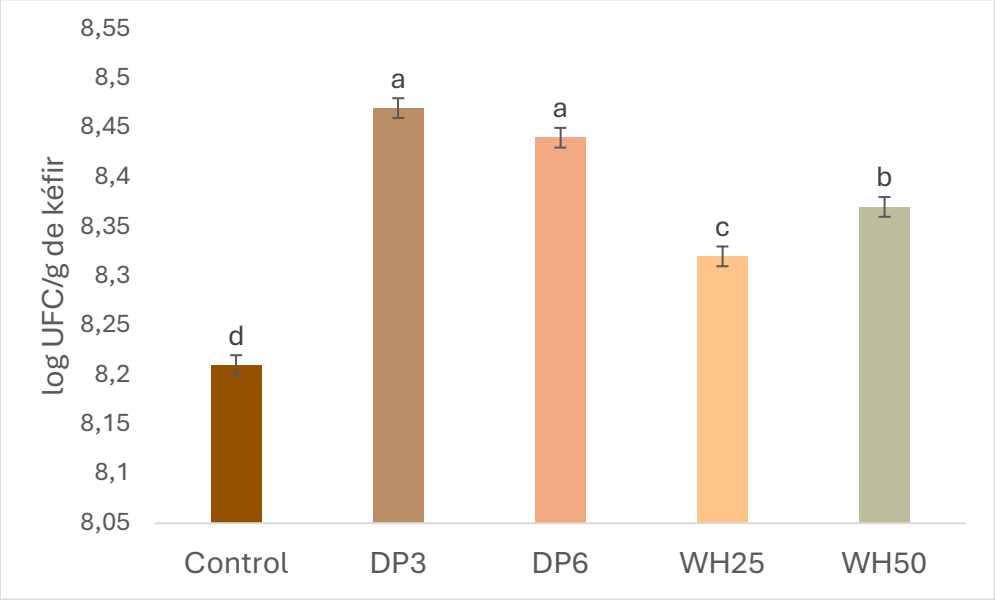


Figura 29. Recuento de *Lactobacillus* spp. presentes en los kéfires de cabra fortificados con pasta de dátil.
a-d Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

En cuanto al análisis sensorial, las muestras de kéfir que contenían suero de queso como sustituto de la leche fueron excluidas de la evaluación debido a que el suero provocó una separación de fases, lo que derivó en la pérdida de su aspecto

normal, considerándose así inadecuado para el estudio. Los evaluadores estudiaron 6 atributos (figura 30), de los cuales solo se encontraron diferencias significativas en uno de ellos. La acidez obtuvo la puntuación más baja en la muestra control, mientras que la muestra con un 6% de pasta de dátil (DP6) obtuvo la puntuación más alta ($p < 0,05$). El resto de los atributos (color, olor, sabor, dulzor y viscosidad) no mostraron diferencias significativas ($p > 0,05$).

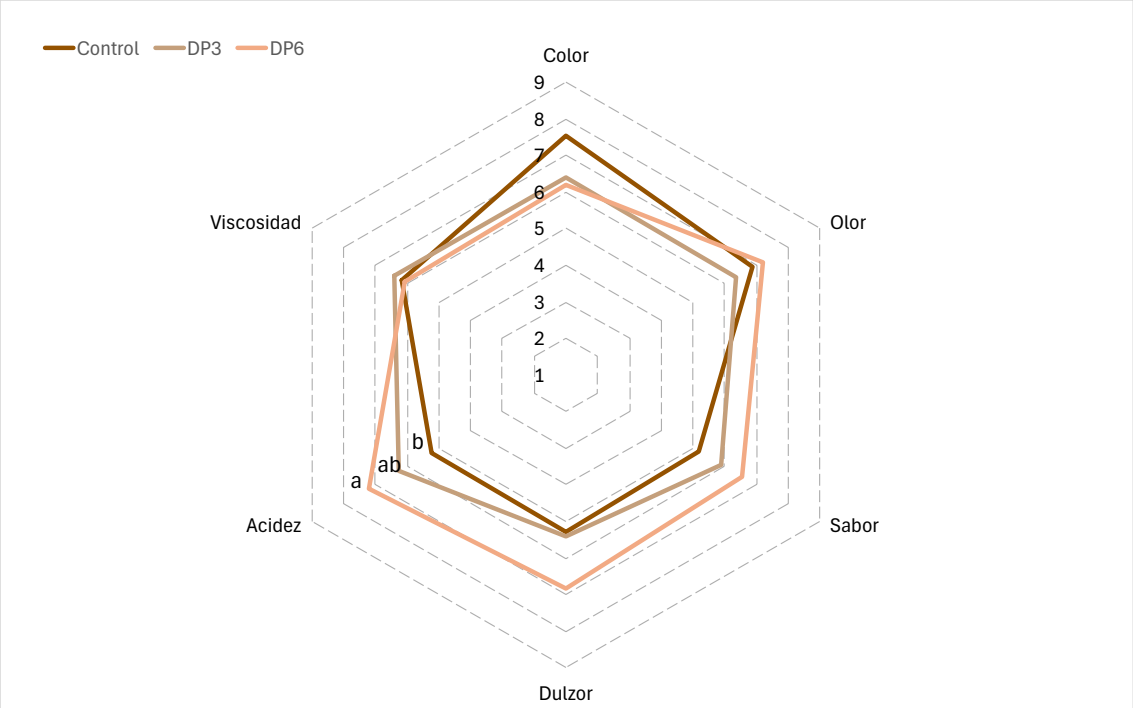


Figura 30. Evaluación sensorial de los kéfires fortificados con pasta de dátil Confitera.
a-b Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

Sin embargo, cabe destacar que el kéfir con un 6% de pasta de dátil (DP6), además de en la acidez, también obtuvo las puntuaciones más altas en el olor, el sabor y el dulzor.

Debido a esto, en términos de aceptabilidad general (figura 31) se puede observar que aunque las diferencias no fueron estadísticamente significativas ($p > 0,05$), DP6 fue la muestra que más gustó a los evaluadores, por lo que la incorporación de dátil, además de no interferir en el proceso de elaboración de los kéfires, han demostrado que son una opción viable tanto nutricional como sensorialmente, siendo a su vez una forma de diversificar la oferta de productos lácteos enriquecidos y funcionales.

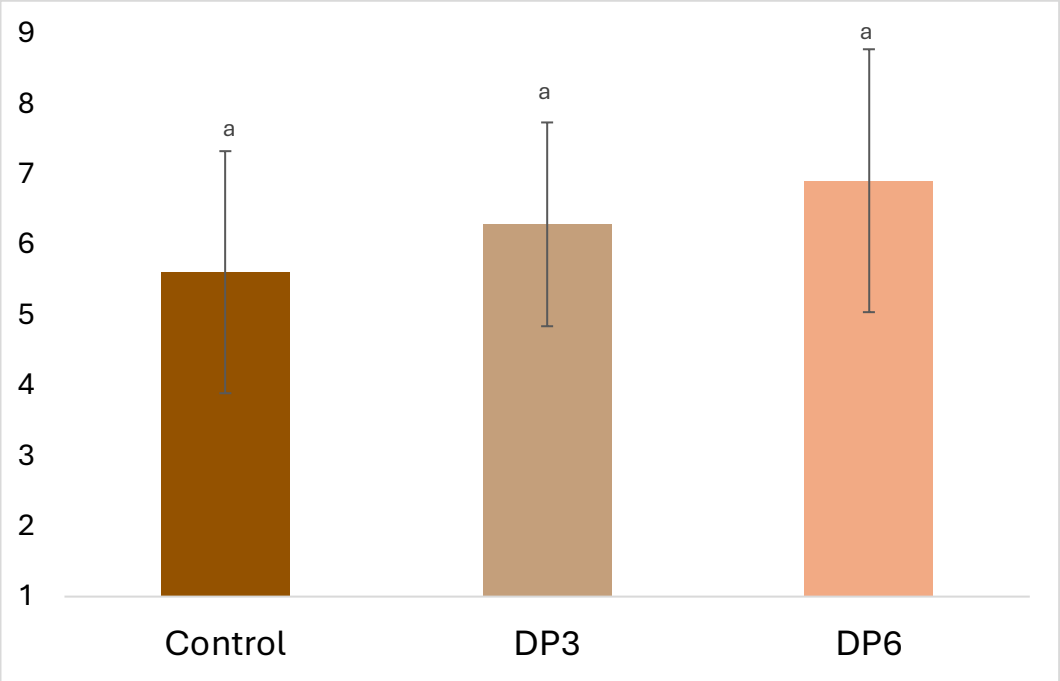


Figura 31. Aceptación general de los kéfires de cabra fortificados con pasta de dátil Confitera. Misma letra encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad.

4.5. PERFIL, ESTABILIDIDAD Y BIOACCESIBILIDAD DE COMPUESTOS FENÓLICOS PRESENTES EN LA PASTA DE DÁTIL Y EL QUESO FRESCO DE CABRA ENRIQUECIDO. EVALUACIÓN ANTES Y DESPUÉS DE LA DIGESTIÓN *IN VITRO*

4.5.1. Perfil de (poli)fenoles en la pasta de dátil Confitera antes y tras la digestión *in vitro*

Tras el estudio del perfil de (poli)fenoles – tanto fracción libre como unida – de la pasta de dátil Confitera se detectaron cincuenta polifenoles, de los cuales, trece fueron confirmados con sus respectivos estándar y el resto tentativamente identificados, comparando sus tiempos de retención y espectros de absorción UV-Vis con los estándares disponibles y la bibliografía publicada (tabla 34), excepto los compuestos n.º 10 y 34, que no se identificaron. Esto fue posible debido a que cada subfamilia de polifenoles presenta un espectro de absorbancia característico y eluyen en orden decreciente de polaridad, por ejemplo, los ácidos fenólicos y los diglucósidos de flavonoides eluyen primero, seguidos de los monoglucósidos, los monoglucósidos acilados y, por último, las agliconas libres (Farag et al., 2014).

Tabla 34. Perfil de compuestos polifenólicos detectados en la pasta de dátil Confitera antes de la digestión *in vitro*.

N.º	Rt (min)	Fr.	Ʌ máx (nm)	Identificación provisional	Patrón para cuantificar
1	7.9	B	236 280	Flavan-3-oles	Catequina
2	11.2	F	242 294sh 318	Derivado Hidroxibenzoico 1	Vanillina
3	11.8	F	244 292sh 314	Glicósido de ácido cafeico	Ácido Caféico
4	12.1	F	242 290sh 320	Glicósido del ácido cafeoilquínico	Chl
5	12.7	F	242 292 318	Derivado Hidroxibenzoico 2	Vanillina
6	12.7	B	236 274 406 474	Derivado de antocianina 1	Pel-3-glu
7	12.9	F	242 290sh 322	Ácido cafeoilsiquímico 1	Chl
8	13.7	B	236 280 310	Glicósido de vanillina 1	Vanillina
9	14.7	F	254 348	Triglicósido de quercetina	Rutina
10	14.8	B	236 320 458	Desconocido	-
11	14.9	F	246 292sh 326	Ácido Clorogénico*	Chl
12	15.2	B	236 280 310	Glicósido de vanillina 2	Vanillina
13	15.3	B	236 280	Catequina*	Catequina
14	15.6	B	236 280	Proantocianidina 1	PAC B2
15	17.3	F	266 338	Glicósido de apigenina	Api-7-glu
16	17.3	F	244 284sh 324	Ácido cafeoilquínico 2	Chl
17	17.6	F	242 290sh 324	Ácido cafeoilquínico 3	Chl
18	17.8	B	236 280	Proantocianidina 2	PAC B2
19	17.9	B	236 280	Epicatequina*	Epicatequina
20	18.1	F	242 300sh 326	Ácido Caféico*	Ácido Caféico
21	18.2	B	236 274 414 474	Derivado de antocianina 2	Pel-3-glu
22	18.4	B	236 280	Proantocianidina 3	PAC B2

23	18.9	F	244 sh286 322	Ácido cafeoilquínico 4	Chl
24	19.4	F	244 sh286 324	Ácido cafeoilquínico 5	Chl
25	19.6	B	240 280	Proantocianidina 4	PAC B2
26	20.0	F	254 358	Diglicósido de quercetina 1	Rutina
27	20.7	F	258 360	Diglicósido de quercetina 2	Rutina
28	21.0	B	238 272 416 472	Derivado de antocianidina	Pel-3-glu
29	21.1	B	236 278	Proantocianidina 5	PAC B2
30	22.1	F	256 358	Diglicosilato de quercetina 3	Rutina
31	22.3	F	256 358	Quercetina-3-rutinósido (Rutina)*	Rutina
32	22.5	B	236 292sh 330	Hexósido de flavanona	Naringina
33	23.7	F	256 264sh 362	Quercetina-3-β-D-glucósido*	Que-3-glu
34	24.1	B	238 270 332 482	Desconocido	-
35	24.1	B	240 278	Catequina-3-gallato*	Cat-3-gal
36	24.6	F/B	246 286sh 324	Ácido ferúlico*	Ácido ferúlico
37	24.9	F	256 358	Glicósido de quercetina 1	Que-3-glu
38	25.4	F	256 358	Glicósido de quercetina 2	Que-3-glu
39	25.9	F	264 354	Isorhamnetina-3-O-glucósido *	Iso-3-glu
40	26.0	F	254 266 350	Diosmetina-7-O-rutinósido (Diosmina)*	Diosmina
41	26.3	F	254 356	Quercetina-3-ramnósido (Quercitrina)*	Quercitrina
42	26.9	B	236 276 452	Derivado de antocianidina 4	Pel-3-glu
43	28.1	F	254 362	Glicósido de isorhamnetina	Iso-3-glu
44	28.7	F	252 268 348	Glicósido de crisoeriol 1	Diosmina
45	29.4	F	252 268 346	Glicósido de crisoeriol 2	Diosmina
46	32.9	B	242 300sh 326	Derivado del ácido cafeico	Ácido Cafeico
47	33.8	F/B	256 370	Quercetina*	Quercetina*
48	38.0	F	252 268 348	Crisoeriol	Diosmina

49	38.5	F	253 266 348	Luteolina metilada	Diosmina
50	38.7	B	266 366	Kaempferol*	Kaempferol

Rt: tiempo de retención; Fr.: tipo de fracción; B: unido insoluble; F: libre soluble;
Chl: Ácido Clorogénico; Proantocianidina B2: PAC B2; Apigenina-7-glucósido: Api-7-glu; Isorhamnetina-3-O-glucósido: Iso-3-glu; Quercetina-3-β-D-glucósido: Que-3-glu; Pelargonidina 3-O-β-glucopiranosida: Pel-3-glu; Catequina-3-galato: Cat-3-gal.*Compuesto confirmado por estándar.

De todos los compuestos detectados, treinta de ellos fueron observados en la fracción libre, siendo esta la mayoritaria. Entre ellos predominaron los ácidos hidroxicinámicos y derivados (diez compuestos), flavonoles (nueve compuestos) y flavanonas (seis compuestos). Mientras, en la fracción ligada destacaron los flavan-3-oles (diez compuestos), siendo la quercetina y el ácido ferúlico los únicos compuestos detectados en ambas fracciones. Por otro lado, se encontraron compuestos libres solubles (glucósido de crisoeriol 1, ácido cafeico y ácido cafeoilquínico 1) (tabla 35).

Tabla 35. Contenido individual de polifenoles y cantidad total de compuestos (µg/g p.s.) de la pasta de dátil Confitera antes y después de la digestión *in vitro*.

Subfamilia	Compuestos		Sin digerir	Digerido
Derivados del ácido hidroxibenzoico	Derivado hidroxibenzoico 1	F	0,90±0,12^{a}	0,81±0,02^{a}
	Derivado hidroxibenzoico 2	F	2,47±0,20^{a}	2,65±0,14^{a}
	Glicósido de vanillina 1	B	0,51±0,13^{b}	3,93±0,36^{a}
	Glicósido de vanillina 2	B	3,06±0,37^{b}	22,69±2,78^{a}
Ácidos hidroxicinámicos y derivados	Glicósido de ácido cafeico	F	1,19±0,07^{a}	0,70±0,10^{b}
	Glicósido del ácido cafeoilsiquímico	F	7,16± 0,84^{a}	7,29±0,85^{a}
	Ácido cafeoilquínico 1	F	8,50±0,58^{a}	5,03±0,2^{b}
	Ácido clorogénico	F	3,21±0,29^{a}	2,66±0,19^{a}
	Ácido cafeoilquínico 2	F	1,12±0,09	ND
	Ácido cafeoilquínico 3	F	3,01±0,34	ND
	Ácido cafeico	F	8,78±0,90^{b}	11,67±0,49^{a}
	Ácido cafeoilquínico 4	F	3,49±0,18^{b}	4,32±0,27^{a}
	Ácido cafeoilquínico 5	F	3,64±0,29^{b}	9,79±1,09^{a}
	Ácido ferúlico	F	0,38±0,04^{b}	0,62±0,07^{a}
		B	5,86±0,48^{b}	15,43±0,4^{a}
	Derivado del ácido cafeico	B	ND	1,16±0,11
Flavonoles	Triglicósido de quercetina	F	ND	0,60±0,11
	Diglicósido de quercetina 1	F	ND	0,56±0,02

	Diglicósido de quercetina 2	F	ND	0,38±0,01
	Diglicósido de quercetina 3	F	1,43±0,21[b]	1,57±0,14[a]
	Quercetina-3-rutinósido	F	1,65±0,11[a]	1,48±0,04[a]
	Quercetina-3-β-D-glucósido	F	1,89±0,21[a]	1,16±0,17[b]
	Glicósido de quercetina 1	F	2,07±0,02[a]	2,29±0,34[a]
	Glicósido de quercetina 2	F	3,60±0,33[a]	2,78±0,32[a]
	Isorhamnetina-3-O-glucósido	F	0,64±0,06[a]	0,33±0,07[b]
	Quercetina-3-rhamnósido	F	1,26±0,14[a]	0,85±0,28[a]
	Glicósido de isorhamnetina	F	1,58±0,17[a]	0,31±0,05[b]
	Quercetina	F	0,60±0,09	ND
		B	1,15±0,27[a]	1,46±0,25[a]
	Kaempferol	B	ND	0,17±0,04
Flavonas	Glicósido de apigenina	F	1,13±0,11[a]	0,95±0,05[a]
	Diosmetina 7-O-rutinósido	F	4,38±0,32[a]	2,25±0,19[b]
	Glicósido de crisoeriol 1	F	16,62±1,16[a]	4,59±0,72[b]
	Glicósido de crisoeriol 2	F	1,42±0,04[b]	2,34±0,16[a]
	Luteolina metilada	F	1,55±0,08	ND
	Crisoeriol	F	2,81±0,25	ND
Flavan-3-oles	Derivado de flavan-3-ol	B	11,44±3,10[b]	40,65±5,41[a]
	Catequina	B	35,25±2,26[a]	21,76±5,73[b]
	Proantocianidina1	B	14,91±2,49[a]	16,64±0,61[a]
	Proantocianidina 2	B	19,02±1,94[a]	12,43±0,31[b]
	Proantocianidina 3	B	19,59±3,47	ND
	Epicatequina	B	10,77±0,7[b]	34,87±2,01[a]
	Proantocianidina 4	B	61,84±5,19[a]	31,45±3,04[b]
	Proantocianidina 5	B	251,67±79,18	ND
	Catequina-3-gallato	B	17,39±2,09	ND
Otros flavonoides	Derivado de antocianina 1	B	ND	11,00±0,47
	Derivado de antocianina 2	B	0,30±0,04[b]	2,10±0,09[a]
	Derivado de antocianina 3	B	1,43±0,25[b]	38,07±0,30[a]
	Derivado de antocianina 4	B	ND	1,93±0,39
	Flavanona	B	13,86±1,19[b]	111,62±8,06[a]
	Total, (poli)fenoles	F	86,88±2,12[a]	68,79±1,61[b]
		B	468,05±85,1[a]	367,36±18,19[a]
	Total, Ácidos hidroxicinámicos	F	40,88±2,45[a]	42,78±1,49[a]
		B	5,86±0,48[b]	16,6±0,39[a]
	Total, Ácidos hidroxibenzoicos	F	3,37±0,26[a]	3,46±0,12[a]

		B	3,57±0,45[b]	26,62±2,87[a]
	Total, Flavonoles	F	14,72±1,23[a]	12,42±1,41[a]
		B	1,15±0,27[a]	1,62±0,22[a]
	Total, Flavonas	F	27,91±1,35[a]	10,13±1,06[b]
	Total, Flavan-3-oles	B	441,89±83,33[a]	157,81±11,93[b]

[a-b] Diferentes letras en la misma fila indican diferencias significativas. ND: no detectado.

Entre los ácidos hidroxicinámicos detectados, el ácido cafeico, el ácido ferúlico y el ácido clorogénico se confirmaron mediante patrón estándar. Los demás compuestos se identificaron provisionalmente como conjugados de ácido cafeico o ácido p-cumárico. Además, los compuestos que mostraron una disminución en su λ máximo en comparación con el ácido cafeico o el ácido clorogénico (318-320 nm, en lugar de 324-328 nm) y el tiempo de elución, se han propuesto como compuestos glicosilados, tal y como han informado muchos autores (Hammouda et al., 2013; Gazi et al., 2024).

Mansouri et al. (2005), también detectaron la presencia de compuestos como isómeros de ácido 5-O- cafeoilquínico, derivados del ácido cafeico, ácido hidrocafeico, ácido p-cumárico, ácido ferúlico, ácido sináptico, derivados del ácido cinámico y ácido cumaroquinico en la variedad Deglet Nour de palmeras datileras de Argelia. Hammouda et al. (2013) detectaron hexóxidos del ácido cafeoilquínico y otros hidrocinamatos, también en la variedad Deglet Nour.

En la variedad Medjool, Khallouki et al. (2018) detectaron ácidos mono y di cafeoilquínico y glucósidos de ácido cafeico y ácido ferúlico. Además, Alfaro-Viquez et al. (2018) informaron que los principales polifenoles observados en los dátiles Medjool y Deglet Noor eran ácidos hidroxicinámicos, y señalaron la presencia de ácidos cafeico, p-cumárico y ferúlico, así como derivados del ácido cinámico.

También se identificaron flavonoides, siendo principalmente quercetina, y luteolina glicosilados y metilados, seguidos por rutina, quercetina-β-D-glucósido, quercitrina, isorhamnetina-3-O-glucósido y diosmetina. Farag et al. (2014) informaron de la presencia de veinte flavonoides en diferentes variedades de dátiles egipcios, siendo principalmente flavanoles y flavonas. Por otro lado, Nematallah et

al. (2018) reportaron diecinueve flavonoides en frutos de dátiles de la variedad Ajwa en la fase Tamar. Además, se ha podido comparar con otros estudios que el estado de maduración de los frutos interfiere en el contenido de compuestos fenólicos, por ejemplo, en los dátiles Deglet Noor en etapa khalal, también se han descrito 13 glucósidos flavonoides de luteolina, quercetina y apigenina. Además, en 21 variedades de frutos de palmera datilera de Egipto en - etapa de maduración Rutab - se han descrito 9 flavonoles y 9 flavonas (Hammouda et al., 2013).

El mayor contenido en polifenoles se encontró en la fracción insoluble, siendo el 87% del contenido total, destacando principalmente la proantocianidina 4 y 5. Estos resultados coinciden con los reportados por Hammouda et al. (2013) donde mostraron que los polímeros de procianidina representaron el 80% del total de polifenoles en dátiles Deglet Nour en fase de maduración tamar. Mientras, el contenido total de polifenoles solubles fue alrededor de 8,5 mg/100 g de peso fresco en la pasta de dátil Confitera. Otros autores reportaron valores de entre 10,9-42,20 mg/100 g de peso seco de dátiles marroquíes (Alahyane et al., 2019) o entre 25,1-33,9 mg/100 g de peso seco de dátiles Boufeggous y Medjoul (Noutfia et al., 2025) ambos en fase tamar. Estas diferencias pueden deberse a que la pasta de dátil Confitera estudiada no se ha sometido al secado al sol, proceso habitual que se lleva a cabo con los dátiles para prolongar su vida útil (Jaouhari et al., 2024).

Por otro lado, cabe destacar que estos resultados obtenidos sufrieron una serie de modificaciones tras el proceso de digestión buco-gastrointestinal al que se sometió dicha pasta de dátil Confitera. Uno de los cambios más destacados fue que de los veintisiete (poli)fenoles solubles detectados en la pasta sin digerir, cinco de ellos – ácidos cafeoilquínico 2 y 3, quercetina, luteolina metilada y crisoeriol – no fueron observados tras la digestión *in vitro*, lo cual pudo deberse, probablemente, a sus bajas concentraciones iniciales. En cambio, algunos flavanoles – triglicósido de quercetina y diglicósidos de quercetina 1 y 3 – solo se detectaron tras la digestión, lo que sugiere que dicho proceso favoreció su liberación. En cuanto a la fracción insoluble unida se observó un patrón similar. Las proantocianidinas 3 y 5 no fueron detectadas tras la digestión. Por el contrario, el

proceso de digestión dio lugar a nuevos compuestos, destacando un derivado del ácido hidroxicinámico, el kaempferol y dos derivados de la antocianina.

De manera general, los (poli)fenoles mostraron una alta estabilidad durante todo el proceso, por lo que la mayoría de compuestos – por ejemplo, glucósido del ácido cafeoilquínico, el ácido clorogénico, la quercetina-3-rutinósido, la quercetina-3-ramnósido y el glucósido de apigenina - no sufrieron cambios significativos en su concentración antes y después de la digestión ($p > 0,05$). Además, compuestos solubles, como el ácido cafeico, el diglucósido de quercetina 3, ácidos cafeoilquínico 4 y 5 y glicósido de criseriol 2, y compuestos insolubles unidos, como el ácido ferúlico, la epicatequina, la flavanona y la antocianina 1 y 4, se detectaron incluso en concentraciones más altas en las muestras digeridas ($p < 0,05$), lo que sugiere un aumento de la liberación o la transformación durante la digestión. Sin embargo, compuestos como el glicósido crisoeriol 1 y el ácido cafeoilquínico 1, mayoritarios en la pasta de dátil Confitera sin digerir, mostraron una marcada disminución tras la digestión *in vitro*. Estos resultados concuerdan con los obtenidos por otros autores. Djaoudene et al. (2021) detectaron una disminución de los flavonoles en la pulpa de dátil tras la digestión, mientras, el ácido cafeico aumentó su concentración. Por otro lado, Kamal et al. (2023) identificaron rutina, ácido cafeico y ácido 4-hidroxibenzoico en muestras digeridas de cuatro variedades de dátiles, los cuales no fueron detectados en las muestras no digeridas. Además, el aumento de los polifenoles solubles pudo deberse a la presencia de formas conjugadas solubles (esterificadas o glicosiladas), que no fueron extraídas por la metodología inicial, pero que se liberaron en el tracto digestivo tras la hidrólisis enzimática. Además, diversos estudios como los realizados por Peters et al. (2010) sobre la biodisponibilidad y la bioaccesibilidad de los polifenoles del té verde tanto *in vivo* como *in vitro*, reportaron que la presencia de sacarosa puede interferir en la unión de los flavan-3-oles a otros componentes del té verde, como la cafeína, lo que mejora la solubilidad de las catequinas en el tracto gastrointestinal mejorando su bioaccesibilidad. Autores como Viuda-Martos et al. (2018) demostraron que la fibra puede atrapar físicamente los polifenoles solubles, proporcionar una protección adicional y mejorar potencialmente su estabilidad durante la digestión.

Teniendo en cuenta los resultados anteriormente expuestos, la pasta de dátil Confitera es un producto de alto valor añadido, rico en fibra dietética y azúcares, compuestos que pueden ayudar a proteger los (poli)fenoles durante el proceso de digestión gastrointestinal.

4.5.2. Perfil de (poli)fenoles del queso fresco de cabra enriquecido con pasta de dátil Confitera antes y tras la digestión *in vitro*

Tras el estudio del perfil de (poli)fenoles en las tres formulaciones de queso fresco de cabra elaborados, cabe destacar, como era de esperar, que en el queso control – es decir, queso sin adición de pasta de dátil Confitera – no se detectaron (poli)fenoles. En cambio, en las formulaciones enriquecidas con pasta de dátil (DPC-4 y DPC-8) se observó un total de veintidós compuestos, de los cuales diecisiete en forma libre y cinco en forma insoluble unida (tabla 36). De entre los compuestos solubles, destacó la presencia del glicósido de crisoeriol 1, el ácido cafeico y el ácido cafeoilquínico 1, coincidiendo con los detectados en la pasta de dátil. También se pudo observar que el queso es más rico en flavonoles que la pasta de dátil, en la cual destacaron los hidroxicinámicos. Esto puede deberse a las diferencias en la solubilidad y afinidad de los compuestos fenólicos con las proteínas de la leche y las posibles pérdidas en el suero. Autores como Mangiapelo et al. (2025) reportaron que el ácido clorogénico se redujo en el queso enriquecido con orujo. Además, se ha informado de que el ácido clorogénico tiene interacciones no covalentes con la β-lactoglobulina de la leche (una proteína presente en la leche de suero) (Ren et al., 2023), lo que podría favorecer dicha disminución. Ciertos estudios como el realizado por Helal et al. (2018) sobre la estabilidad de los (poli)fenoles en yogures enriquecidos con café, reportó una reducción de los ácidos hidroxicinámicos en la leche de vaca.

En cuanto a la fracción insoluble, los nueve flavan-3-oles identificados en la pasta de dátil, solo se informó la presencia de proantocianidina 4 en el queso enriquecido. En cambio, se detectaron dos derivados de vanillina, ácido ferúlico y derivados de antocianina. Sin embargo, teniendo en cuenta que estos compuestos se han detectado en fracciones insolubles, otros procesos deben estar

contribuyendo a su pérdida. Además, la pérdida de flavan-3-oles se ha descrito anteriormente en otras matrices alimentarias, como el paté de hígado de cerdo enriquecido con harina de caqui, que se encontraba entre los diez compuestos detectados en la harina vegetal, solo uno fue identificado en la matriz de carne enriquecida (Lucas-González et al., 2020).

En la tabla 36 se puede observar las modificaciones detectadas en el perfil (poli)fenólico del queso fresco tras la digestión gastrointestinal *in vitro*. De los diecisiete (poli)fenoles solubles detectados en las muestras DCP-4 y DCP-8 no digeridas, solo se identificaron cuatro compuestos tras la digestión: glucósido de ácido cafeoilquínico, ácido cafeico, ácido cafeoilquínico 5 y glucósido de quercetina 1, los cuales mostraron una reducción significativa de su concentración tras dicho proceso ($p < 0,05$).

Tabla 36. Perfil de (poli)fenoles (µg/g) de quesos frescos de cabra enriquecidos con pasta de dátil Confitera, antes y después de la digestión *in vitro*.

		DPC-4 sin digerir	DPC-4 digerido	DPC-8 sin digerir	DPC-8 digerido
Derivado hidroxibenzoico 1	F	2,15±0,21^{b}	ND	5,87±0,43^{a}	ND
Derivado hidroxibenzoico 2	F	4,20±0,48^{b}	ND	8,50±0,42^{a}	ND
Glicósido de vanillina 1	B	4,46±0,33^{b}	18,94±2,68^{c}	4,91±0,93^{b}	30,27±2,88^{a}
Glicósido de vanillina 2	B	33,55±10,36^{b}	102,71±3,60^{c}	32,43±14,86^{b}	241,30±3,50^{a}
Glicósido del ácido cafeoilquínico	F	17,35±2,10^{b}	3,46±0,12^{c}	36,00±3,19^{a}	4,00±0,08^{c}
Ácido clorogénico	F	2,94±0,69^{b}	ND	10,29±0,02^{a}	ND
Ácido cafeico	F	32,89±0,39^{b}	16,03±0,22^{c}	54,51±2,80^{a}	17,46±0,17^{c}
Ácido cafeoilquínico 5	F	19,49±4,02^{b}	4,66±0,45^{c}	49,34±6,63^{a}	10,51±0,69^{c}
Ácido ferúlico	F	16,25±2,94^{b}	ND	27,23±0,07^{a}	ND
	B	5,36±0,37^{c}	55,75±3,3^{b}	10,53±0,11^{c}	118,60±3,55^{a}
Diglicósido de quercetina 3	F	8,10±0,90^{b}	ND	15,68±1,72^{a}	ND
Quercetina-3-rutinósido (rutina)	F	9,24±1,84^{b}	ND	15,36±1,50^{a}	ND
Quercetina-3-β-D-glucósido	F	6,25±0,22^{a}	ND	9,63±2,27^{a}	ND
Glicósido de quercetina 1	F	12,92±1,92^{b}	3,49±0,08^{c}	24,31±1,52^{a}	4,90±0,20^{c}

Glicósido de quercetina 2	F	16,70±3,55^{b}	ND	29,93±0,94^{a}	ND
Quercetina-3-ramnosida (Quercitrina)	F	4,92±0,37^{b}	ND	8,54±0,34^{a}	ND
Glicósido de isorhamnetina	F	2,75±0,20^{b}	ND	5,71±1,06^{a}	ND
Glicósido de apigenina	F	3,99±0,34^{b}	ND	7,92±0,51^{a}	ND
Diosmetina 7-O-rutinósido (diosmina)	F	10,68±0,78^{b}	ND	25,69±0,54^{a}	ND
Glicósido de crisoeriol 1	F	34,78±5,26^{b}	ND	70,23±8,16^{a}	ND
Flavanona	B	ND	870,05±8,93^{b}	ND	1150,58±70,54^{a}
Proantocianidina 4	B	251,63±15,89^{a}	ND	284,57±9,60^{a}	ND
Derivado de antocianina 1	B	ND	Tr	ND	Tr
Derivado de antocianina 3	B	21,32±0,13^{a}	65,61±14,82^{a}	30,19±6,38^{a}	98,24±50,72^{a}
Derivado de antocianina 4	B	ND	38,29±27,08	ND	40,11±0,71
Total de (poli)fenoles	F	205,6±24,58^{b}	27,65±0,6^{c}	404,73±23,30^{a}	36,86±1,02^{c}
	B	316,33±10,68^{c}	1220,2±64,12^{b}	362,63±12,33^{c}	1700,1±32,74^{a}
Ácidos hidroxicinámicos totales	F	88,92±9,48^{b}	24,16±0,57^{c}	177,36±10,67^{a}	31,96±0,82^{c}
	B	5,36±0,37^{c}	55,75±3,3^{b}	10,53±0,11^{c}	118,6±3,55^{a}
Ácidos hidroxibenzoicos totales	F	6,35±0,63^{b}	ND	14,37±0,70^{a}	ND
	B	38,02±10,03^{c}	121,65±0,00^{b}	37,34±15,64^{c}	271,57±5,97^{a}
Flavonoles totales	F	60,88±8,38^{b}	3,49±0,08^{c}	109,16±7,65^{a}	4,90±0,20^{c}
Flavonas totales	F	49,45±6,35^{b}	ND	103,84±8,29^{a}	ND
Total de flavan-3-oles	B	251,63±15,89^{a}	ND	284,57±9,60^{a}	ND

$^{a-c}$ Diferentes letras entre columnas significan diferencias significativas. ND: no detectado. Tr: traza. F: fracción soluble libre. B: fracción insoluble unida.

Por otro lado, cabe destacar que el glicósido de crisoeriol 1, el flavonoide soluble más abundante en los quesos frescos de cabra enriquecidos con pasta de dátil Confitera sin digerir, ya no se detectó en las muestras digeridas, lo que refleja su inestabilidad. En cuanto a las fracciones insolubles ligadas de los quesos frescos de cabra, todos los (poli)fenoles detectados mostraron concentraciones más altas después de la digestión en comparación con las muestras no digeridas, excepto la proantocianidina 4, que ya no se detectó después de la fase intestinal. Además, se detectaron tres nuevos compuestos —la flavona y los derivados de

antocianina 1 y 4— en ambos quesos de cabra digeridos, pero no en las muestras no digeridas.

Las principales diferencias en las concentraciones de (poli)fenoles entre los dos quesos frescos de cabra enriquecidos con pasta de dátil Confitera (DPC-4 frente a DPC-8) tras la digestión gastrointestinal se observaron solo en los ácidos fenólicos insolubles, donde se encontraron concentraciones significativamente más altas en el DPC-8. Para todos los demás compuestos solubles e insolubles, ambos quesos mostraron niveles similares ($p > 0,05$). Un estudio sobre yogur enriquecido con extracto de semillas de dátiles mostró una alta estabilidad de los polifenoles después de la fase intestinal (Hilary et al., 2020). Aunque el yogur también es un producto lácteo, su matriz y composición difieren considerablemente del queso fresco de cabra, lo que puede explicar las diferencias observadas.

4.5.3. Bioaccesibilidad e índice de colon disponible de la pasta de dátil Confitera y queso fresco de cabra enriquecido con pasta de dátil

La bioaccesibilidad de los polifenoles se refiere a aquellos compuestos liberados de la matriz alimentaria que están disponibles para su transformación y absorción en el torrente sanguíneo. Por el contrario, el índice de disponibilidad en el colon se refiere a los compuestos que permanecen unidos a la matriz alimentaria y pueden ser metabolizados por la microbiota colónica (Lucas-González et al., 2021).

La figura 32 muestra la bioaccesibilidad y el índice de disponibilidad en el colon del contenido total de polifenoles de la pasta de dátil, DPC-4 y DPC-8.

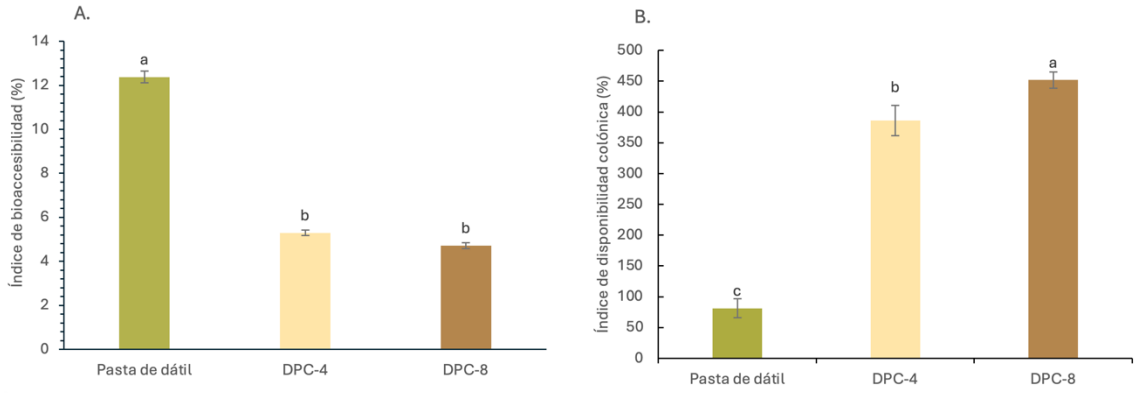


Figura 32. A) índice de bioaccesibilidad total (%) tanto de la pasta de dátil Confitera como del queso fresco de cabra enriquecido con un 4% (DPC-4) y un 8% (DPC-8) de pasta de dátil. B) índice de disponibilidad colónica (%) tanto de la pasta de dátil Confitera como del queso fresco de cabra enriquecido con un 4% (DPC-4) y un 8% (DPC-8) de pasta de dátil.
[a-c]Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

La pasta de dátil Confitera mostró una mayor bioaccesibilidad total de compuestos polifenólicos ($p < 0,05$), mientras que el queso fresco de cabra enriquecido con un 8 % de pasta de dátil mostró un índice de disponibilidad en el colon más alto ($p < 0,05$). No obstante, la bioaccesibilidad global relativamente baja de los (poli)fenoles en la pasta de dátil contrasta con la elevada bioaccesibilidad observada para ciertos compuestos, cuyos índices oscilaron entre el 50% y el 260% (figura 33).

Este hecho podría deberse a que la fracción más importante de la pasta de dátil está constituida por los (poli)fenoles insolubles, los cuales no se liberaron durante la digestión *in vitro*. Estos hallazgos evidencian la influencia de la matriz alimentaria en la bioaccesibilidad y en el índice de disponibilidad colónica de los compuestos (poli)fenólicos. En particular, la estructura compleja del queso fresco de cabra limitó significativamente la liberación y/o estabilidad de estos compuestos a lo largo del proceso de digestión gastrointestinal *in vitro*.

En contraste, cuando la pasta de dátil Confitera se sometió a digestión de manera aislada, sus principales polifenoles mantuvieron una elevada estabilidad y

bioaccesibilidad (Figura 33). Además, estos resultados muestran una tendencia similar a los reportados por otros autores, en los que se observó una mayor bioaccesibilidad de los (poli)fenoles en tomate y pimiento frescos en comparación con los alimentos procesados elaborados a partir de ellos (Lucas-González et al., 2023).

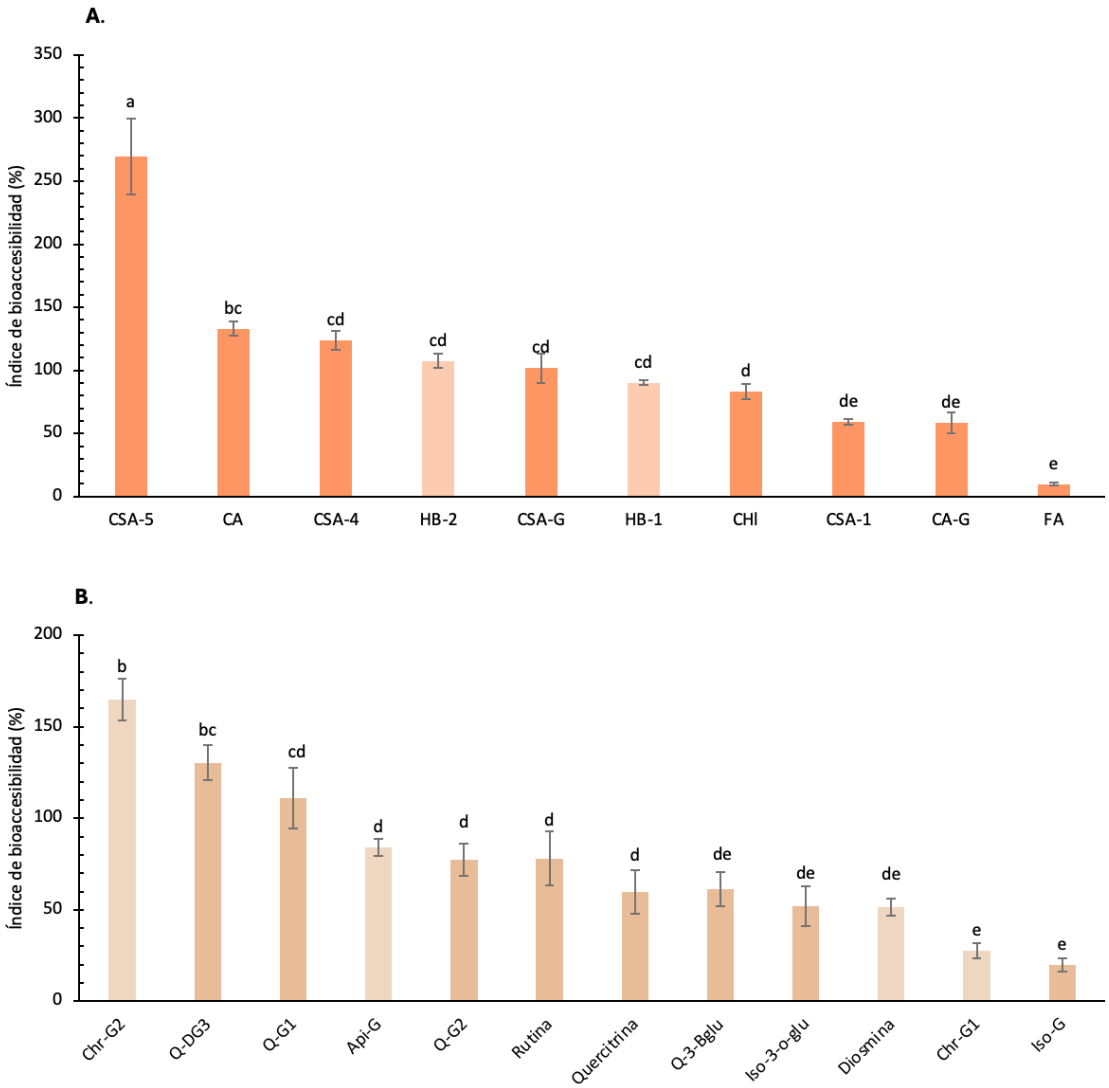


Figura 33. A) índice de bioaccesibilidad de los ácidos hidroxicinámicos e hidroxibenzoicos individuales y derivados de la pasta de dátil Confitera. CSA-5: Ácido cafeoilsiquímico 5; CA: Ácido cafeico; CSA-4: Ácido cafeoilsiquímico 4; HB-2: Derivado hidroxibenzoico 2; CSA-G: Glicósido del ácido cafeoilquínico; HB-1: Derivado hidroxibenzoico 1; CHl: Ácido clorogénico; CSA-1: Ácido cafeoilsiquímico 1; CA-G: Glicósido del ácido cafeico; Fa: Ácido ferúlico.

B) Índice de bioaccesibilidad de los flavonoles individuales y flavonas de las pasta de dátil Confitera. Chr-G2: Glicósido de crisoeriol 2; Q-DG3: Diglicósido de quercetina 3; Q-G1: Glicósido de quercetina 1; Api-G: Glicósido de apigenina; Q-G2: Glicósido de quercetina 2; Q-3 -Bglu: Quercetina-

3-β-D-glucósido (isoquercitrina); Iso-3-o-glu: Isorhamnetina-3-O-glucósido; Chr-G1: Glicósido de crisoeriol 1; Iso-G: Glicósido de isorhamnetina.

[a-e] Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

En relación con los cuatro compuestos bioaccesibles identificados en el queso fresco de cabra enriquecido con pasta de dátil (Figura 34), se observaron diferencias más marcadas en comparación con la propia pasta de dátiles. En esta última, los compuestos glucósido del ácido cafeoilsiquímico, ácido cafeoilsiquímico 5 y glucósido de quercetina 1 presentaron índices de bioaccesibilidad iguales o superiores al 100 %. No obstante, en las muestras de queso enriquecido (DPC-4 y DPC-8), dichos valores se redujeron notablemente, situándose entre el 11,10 % y el 48,78 %. Además, no se detectaron diferencias significativas entre ambos quesos ($p > 0,05$), lo que sugiere que la limitada bioaccesibilidad de los polifenoles procedentes del dátil incorporados en la matriz del queso no dependía de su concentración inicial.

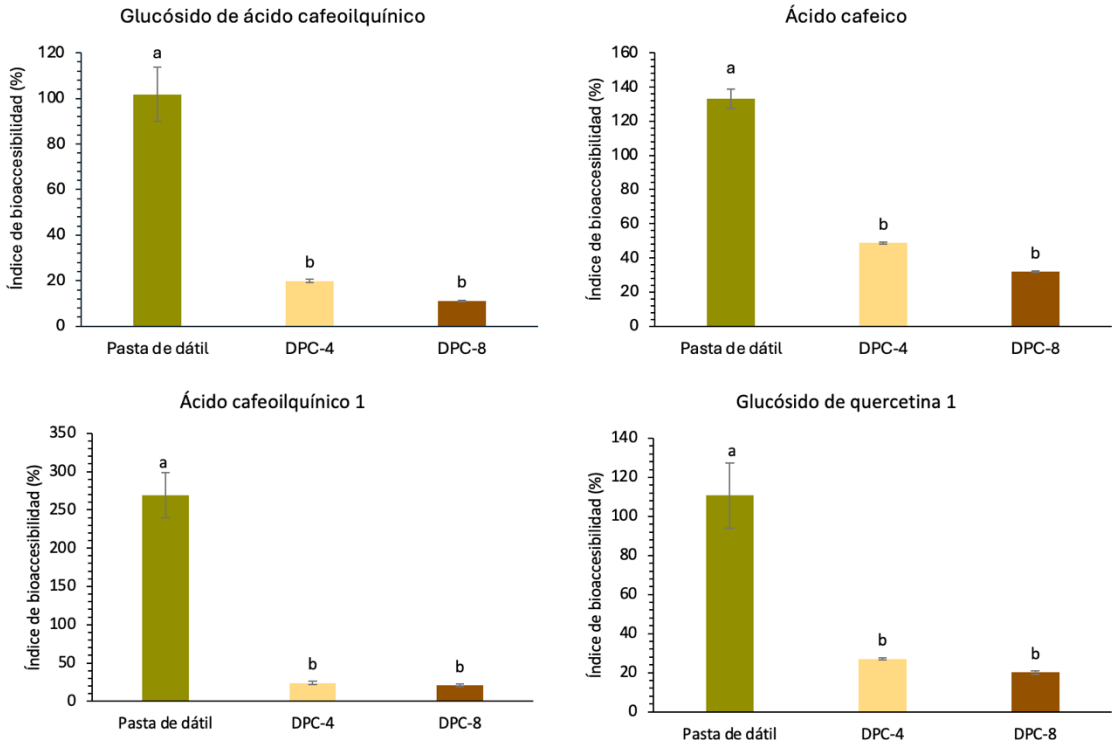


Figura 34. Índice de bioaccesibilidad total (%) de los compuestos (poli)fenólicos tanto de la pasta de dátil Confitera como del queso fresco de cabra enriquecido con un 4% (DPC-4) y un 8% (DPC-8) de pasta de dátil

[a-b] Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

En cuanto al índice de disponibilidad colónica de los (poli)fenoles insolubles individuales (Figura 35), se observaron tendencias diferenciadas entre el queso fresco enriquecido y la pasta de dátil. Para los glucósidos de vainillina 1 y 2, los valores fueron comparables entre las tres muestras ($p > 0,05$). En cambio, el ácido ferúlico mostró un índice de disponibilidad colónica significativamente menor en la pasta de dátil que en los quesos enriquecidos ($p < 0,05$), mientras que el derivado de antocianina 3 alcanzó los valores más elevados en la pasta de dátil ($p < 0,05$). En conjunto, estos resultados indican que, aunque el proceso de digestión *in vitro* promovió un incremento en la liberación de estos compuestos, su comportamiento final estuvo condicionado tanto por la estructura química individual como por la matriz alimentaria en la que se encontraban.

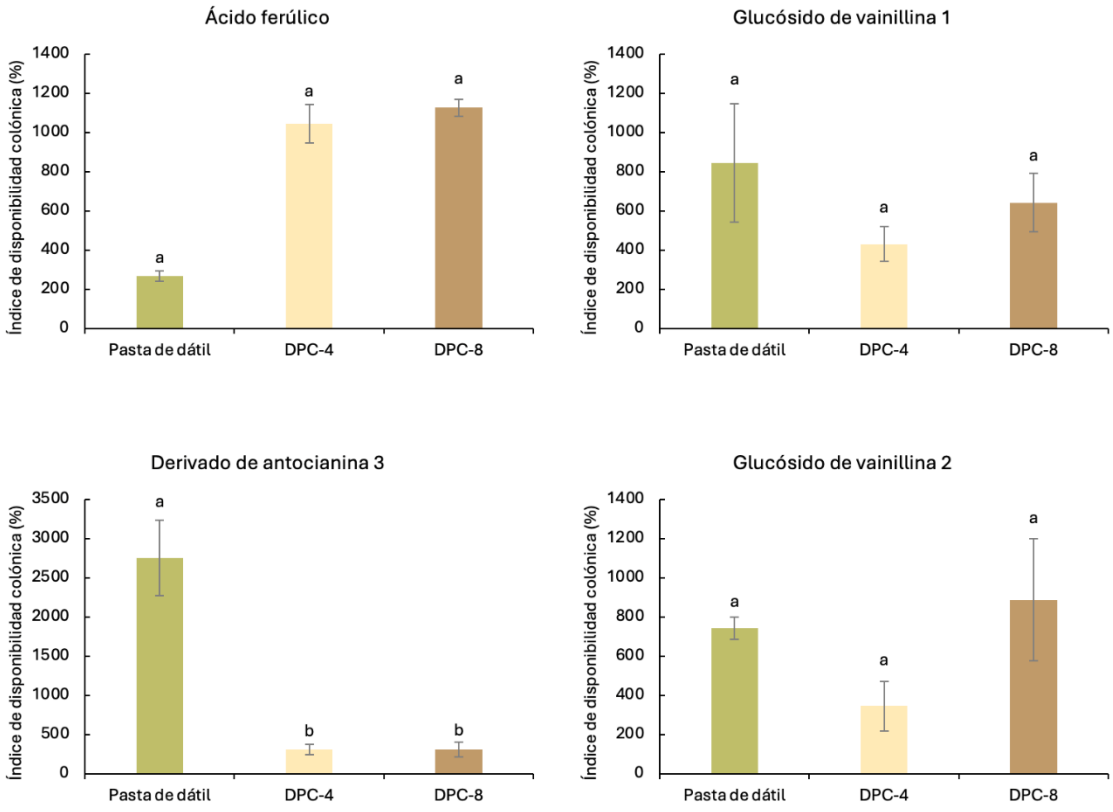


Figura 35. Índice de disponibilidad colónica (%) de los compuestos (poli)fenólicos insolubles tanto de la pasta de dátil Confitera como del queso fresco de cabra enriquecido con un 4% (DPC-4) y un 8% (DPC-8) de pasta de dátil.

[a-b] Diferentes letras encima de cada barra indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad.

CAPÍTULO 5. CONCLUSIONES



5. CONCLUSIONES

5.1. CONCLUSIONES

1. Los resultados obtenidos respaldan la importancia de fomentar el cultivo y la producción de la variedad autóctona de dátil Confitera (*Phoenix dactylifera L.*) en el sureste de España, particularmente en el palmeral de Elche. Este impulso no solo contribuiría al fortalecimiento de la agricultura sostenible y a la preservación de la biodiversidad local, sino que además permitiría aprovechar las ventajas productivas cualitativas de esta variedad frente a la Medjoul, ya que los dátiles Confitera presentan un mayor tamaño y un rendimiento de pulpa superior, cuando se cultivan bajo las condiciones climáticas específicas del ecosistema ilicitano.

2. Nutricionalmente los dátiles frescos del cultivar Confitera destacan por ser fuente de carbohidratos y fibra dietética, siendo los principales azúcares libres la glucosa y la fructosa (azúcares reductores). En su perfil de minerales destacan el potasio y el magnesio. También son una fuente importante de polifenoles (en mayor número y concentración que los Medjoul), principalmente flavonoides, con un notable potencial antioxidante *in vitro*.

3. La valorización de los coproductos de los dátiles frescos (Confitera cv.), generados durante la comercialización e industrialización de los mismos, mediante la aplicación de procesos tecnológicos sencillos y respetuosos con el medio ambiente (remojo, filtración, secado y molienda) permitió la obtención de productos de alto valor añadido (pasta, agua y harina de dátil) con un gran potencial para su uso como productos alimentarios intermedios. Los tres productos obtenidos presentan características fisicoquímicas, nutricionales y biológicas diferentes, aunque destacan por su elevada concentración de azúcares naturales, fibra dietética, minerales esenciales y compuestos bioactivos, especialmente (poli)fenoles, lo que les confiere un importante potencial como fuentes naturales de energía y antioxidantes. La harina de dátil

presentó los niveles más altos de compuestos fenólicos totales, actividad antioxidante, antidiabética y antihipertensiva. La harina y el agua de dátil mostraron un elevado potencial prebiótico a concentraciones adecuadas.

4. La incorporación de estos productos alimentarios intermedios a diferentes matrices lácteas (yogur, queso y kéfir) resultó un proceso tecnológicamente viable, sin necesidad de variar significativamente el proceso original de producción de los mismos. Su incorporación a diferentes concentraciones contribuyó a la fortificación de los productos lácteos.

5. En el caso de los yogures, la adición de harina y pasta de dátil (3% y 6%) aceleró el proceso de acidificación, sin llegar a comprometer las propiedades fisicoquímicas, texturales ni nutricionales, dando lugar a un producto con características similares al yogur convencional. Además, se mejoró la composición nutricional de los yogures, elevando el contenido de minerales (como K, Mn, Cu y Zn), ácidos orgánicos (ácido láctico) y azúcares (glucosa). También se consiguió incrementar el potencial probiótico de los yogures debido a que estos ingredientes estimularon el crecimiento y la estabilidad del cultivo iniciador. La adición de harina produjo modificaciones más pronunciadas en textura, color y sinéresis en comparación con los yogures con pasta de dátil. Durante el almacenamiento, la pasta de dátil redujo la sinéresis de los yogures, manteniendo una calidad fisicoquímica estable. Sensorialmente, todos los yogures fueron bien aceptados por los consumidores, destacando principalmente los yogures con adición de pasta de dátil tanto al 3% como al 6%, como los mejor valorados.

6. El enriquecimiento del queso fresco de cabra con pasta de dátil (4% y 8%) demostró ser un enfoque sostenible, eficaz y tecnológicamente viable. Su incorporación no alteró el proceso de coagulación del queso. Además, los quesos con pasta de dátil mostraron un perfil nutricional mejorado, siendo más notable en el queso con un 8% de adición, donde destacó un incremento en el contenido en minerales (Ca, K, Mg, Na y P), azúcares (lactosa y fructosa) y

ácidos orgánicos (ácido láctico), sin comprometer las propiedades fisicoquímicas y texturales. La pasta de dátil se integró perfectamente en la matriz proteica, conservando la red proteico-lipídica característica del queso fresco de cabra.

7. El enriquecimiento de kéfir de cabra con pasta de dátil (3% y 6%) fue tecnológicamente viable, en cambio, la adición de suero de queso procedente de la elaboración del queso fresco con un 8% de pasta de dátil como sustituto de la leche de cabra (>25 %), disminuyó la viscosidad típica del kéfir, lo que provocó una separación de fases excesiva, comprometiendo de esta forma las características tecnológicas del producto. La adición de pasta de dátiles también mejoró el perfil mineral del kéfir, con un aumento significativo en cuanto al Ca, K, Mg, Na y P. Además, la incorporación de este producto alimentario intermedio aumentó el contenido en fructosa, que no estaba presente en el kéfir control. También se incrementó el potencial probiótico del kéfir con la pasta de dátil, lo que puede estar estrechamente relacionado con el aporte de minerales y compuestos (poli)fenólicos.

8. Durante el estudio de digestión *in vitro*, la pasta de dátil se confirmó como una fuente relevante de (poli)fenoles (50 compuestos). La distribución entre las fracciones libre e insoluble se relacionó con la naturaleza del compuesto: los ácidos hidroxicinámicos, flavonas y flavonoles predominaron en la fracción libre, mientras que los flavan-3-oles y proantocianidinas se asociaron a la fracción unida. La digestión *in vitro* modificó significativamente este perfil fenólico, reduciendo la bioaccesibilidad del crisoeriol glucósido 1 y promoviendo la liberación de nuevos glucósidos flavonoides no presentes en la muestra inicial.

9. Cuando el proceso de digestión *in vitro* se llevó a cabo sobre el queso fresco adicionado con 4% y 8% de pasta de dátil, la matriz láctea influyó notablemente en la estabilidad de los (poli)fenoles de la pasta de dátil. De los veintidós compuestos detectados, la recuperación fue inferior al 60 % en ambas

formulaciones (4 % y 8 %). Los flavonoles solubles mostraron mayor retención en el queso fresco que los ácidos hidroxicinámicos y las flavonas, aunque tras la digestión *in vitro* solo se liberaron cuatro (poli)fenoles en cantidades reducidas, ninguno perteneciente a la familia de los flavonoles. La digestión resultó, no obstante, determinante para incrementar la detección de (poli)fenoles insolubles unidos.

5.2. CONCLUSIONS

1. The results obtained support the importance of promoting the cultivation and production of the native Confitera date variety (Phoenix dactylifera L.) in south-eastern Spain, particularly in the palm grove of Elche. This initiative would not only contribute to strengthening sustainable agriculture and preserving local biodiversity, but would also allow the qualitative production advantages of this variety over Medjoul to be exploited, as Confitera dates are larger and have a higher pulp yield when grown under the specific climatic conditions of the Elche ecosystem.

2. Nutritionally, fresh dates from the Confitera cultivar stand out as a source of carbohydrates and dietary fibre, with glucose and fructose (reducing sugars) being the main free sugars. Potassium and magnesium are notable in their mineral profile. They are also an important source of polyphenols (in greater numbers and concentration than Medjoul dates), mainly flavonoids, with notable antioxidant potential *in vitro*.

3. The valorisation of the by-products of fresh dates (Confitera cv.), generated during their commercialisation and industrialisation, through the application of simple and environmentally friendly technological processes (soaking, filtration, drying and grinding), has enabled the production of high added-value products (date paste, water and flour) with great potential for use as intermediate food products. The three products obtained have different

physicochemical, nutritional and biological characteristics, although they stand out for their high concentration of natural sugars, dietary fibre, essential minerals and bioactive compounds, especially (poly)phenols, which gives them significant potential as natural sources of energy and antioxidants. Date flour had the highest levels of total phenolic compounds, antioxidant, antidiabetic and antihypertensive activity. Date flour and water showed high prebiotic potential at appropriate concentrations.

4. The incorporation of these intermediate food products into different dairy matrices (yoghurt, cheese and kefir) proved to be a technologically viable process, without the need to significantly alter the original production process. Their incorporation at different concentrations contributed to the fortification of dairy products.

5. In the case of yoghurts, the addition of flour and date paste (3% and 6%) accelerated the acidification process without compromising the physicochemical, textural or nutritional properties, resulting in a product with characteristics similar to conventional yoghurt. In addition, the nutritional composition of the yoghurts was improved, increasing the content of minerals (such as K, Mn, Cu and Zn), organic acids (lactic acid) and sugars (glucose). The probiotic potential of the yoghurts was also increased because these ingredients stimulated the growth and stability of the starter culture. The addition of flour produced more pronounced changes in texture, colour and syneresis compared to yoghurts with date paste. During storage, date paste reduced the syneresis of the yoghurts, maintaining stable physicochemical quality. Sensory evaluation showed that all yoghurts were well accepted by consumers, with those containing 3% and 6% date paste being the most highly rated.

6. Enriching fresh goat cheese with date paste (4% and 8%) proved to be a sustainable, effective, and technologically viable approach. Its incorporation did not alter the cheese coagulation process. In addition, cheeses with date paste showed an improved nutritional profile, most notably in cheese with an 8% addition, where there was an increase in the content of minerals (Ca, K, Mg, Na and P), sugars (lactose and fructose) and organic acids (lactic acid), without compromising the physicochemical and textural properties. The date paste integrated perfectly into the protein matrix, preserving the protein-lipid network characteristic of fresh goat's cheese.

7. Enriching goat kefir with date paste (3% and 6%) was technologically feasible, However, the addition of whey from fresh cheese production with 8% date paste as a substitute for goat's milk (>25%) decreased the typical viscosity of kefir, causing excessive phase separation and thus compromising the technological characteristics of the product. The addition of date paste also improved the mineral profile of kefir, with a significant increase in Ca, K, Mg, Na and P. In addition, the incorporation of this intermediate food product increased the fructose content, which was not present in the control kefir. The probiotic potential of kefir was also increased with date paste, which may be closely related to the contribution of minerals and (poly)phenolic compounds.

8. During the *in vitro* digestion study, date paste was confirmed as a significant source of (poly)phenols (50 compounds). The distribution between the free and insoluble fractions was related to the nature of the compound: hydroxycinnamic acids, flavones and flavonols predominated in the free fraction, while flavan-3-ols and proanthocyanidins were associated with the bound fraction. *In vitro* digestion significantly modified this phenolic profile, reducing the bioaccessibility of chrysoeriol glycoside 1 and promoting the release of new flavonoid glycosides not present in the initial sample

9. When the *in vitro* digestion process was carried out on fresh cheese with 4% and 8% date paste added, the milk matrix had a significant influence on the stability of the date paste (poly)phenols. Of the twenty-two compounds detected, recovery was less than 60% in both formulations (4% and 8%). Soluble flavonols showed greater retention in fresh cheese than hydroxycinnamic acids and flavones, although after *in vitro* digestion only four (poly)phenols were released in reduced amounts, none belonging to the flavonol family. Digestion was, however, decisive in increasing the detection of bound insoluble (poly)phenols.

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CAPÍTULO 7. PUBLICACIONES



7.1. PUBLICACIÓN 1

Quality characteristics of fresh date palm fruits of “Medjoul”

and “Confitera” cv. from the southeast of Spain (Elche Palm Grove)

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Quality Characteristics of Fresh Date Palm Fruits of "Medjoul" and "Confitera" cv. from the Southeast of Spain (Elche Palm Grove)

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Abstract: The quality characteristics (physical, techno-functional, and chemical) of date fruits (at the tamar stage) of two cultivars ("Confitera", autochthonous and unknown vs. "Medjoul", widely distributed and well-known), grown in the Southeast of Spain (Elche palm grove) were evaluated in order to reinforce decisions aimed at organizing the production of fresh dates from Elche by selecting the most profitable cultivar. Morphologically, Confitera dates were longer and with higher pulp yield than Medjoul dates (4.58 cm vs. 3.88 cm, and 84% vs. 78%, respectively) ($p < 0.05$). Nutritionally, both dates are a good source of carbohydrates (total sugars (43–48%) and dietary fiber (20–22%)), with small amounts of fat and proteins. The main free sugars in dates from both cultivars were glucose and fructose (reducing sugars). The most abundant mineral found in both date fruits were K, followed by Ca or Mg (depending on the cultivar; Ca in Medjoul and Mg in Confitera). Confitera dates showed a higher total antioxidant activity than Medjoul, corresponding with their higher ($p < 0.05$) content in polyphenolic compounds, mainly flavonoids (catechin and epicatechin predominantly). Confitera dates should be promoted in this region not only for their contribution to sustainable agricultural development and biodiversity, but also for their higher overall quality.

Keywords: date fruit; Confitera; Medjoul; food quality; polyphenol composition; antioxidant properties

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1. Introduction

The date, a fruit of the date palm tree (*Phoenix dactylifera* L.) that originated in the ancient world (dating back to before 4000 B.C.), is of great cultural, social, environmental, livelihood, and economic importance in many countries, being particularly important in arid and semiarid regions with marginal agriculture, where it is one of the pillars on which they are based. In these regions, it has been considered a relevant subsistence crop without misleading its importance as a cultural legacy in Arabic countries and some Islamic ones. From its origin (ancient Mesopotamia or western India), date culture has been spread to other areas of the world such as South Asia, North Africa, around Mediterranean, Australia, Mexico, and United States. All these areas have in common a hot and dry climate (long and hot summer, little rain and low humidity), and have typical characteristics of desert and semi-desert areas that can be found in larger zones due to current climate change [1–3]. It is the case of the Southeast of Spain (Elche, Alicante) where the largest palm grove in Europe and one of the northern and largest in the world (approx. 500 hectares with more than 200,000 palm trees' specimens, whose production in 2020 was 1375 tons) is located [4], declared in 2000 a World Heritage Site by UNESCO.

Traditionally, dates have been a product of exchange and domestic trade, which means that they have been mainly consumed in productive countries, but this has undergone a change over time, and currently there is a very active trade of dates from the production areas to Europe and America. This date export market is essentially supplied by dry and semi-soft dates, which are easier to preserve than fresh dates that are rich in water [5,6]. However, fresh dates are healthier and are most appreciated by current consumers. In addition, although it is believed that more than 2000 cultivars of dates exist [7], only a few have importance due to their agronomic characteristics and fruit quality, being Medjoul cv. one of the most valuable in the European trade. The Medjoul cv. has distinct advantages over other cultivars, such as high yields and quality fruits, huge value in the international market, and great nutritional value [8]. This is the cultivar that has been mainly cultivated in Elche palm grove although during last years a not well-known autochthonous Confitera cv. is being promoted due to it is completely adapted to this particular habitat, ecosystem, and region and also a way to preserve biodiversity. At this time, Medjoul and Confitera cv. are the only cultivars with commercial potential in this area.

Nutritionally, date fruits are an important (and cheap) source of carbohydrates (total sugars: 50–80 g/100 g dw, and dietary fiber: 6.4–11.5 g/100 g dw (mainly insoluble dietary fiber)) with a low content in fats (0.2–0.5 g/100 g dw) and proteins (1.6–4.7 g/100 g dw) [1]. In addition, their content in micronutrients (minerals such as K, Ca, Mg, Na, Mn, Zn and Fe) and vitamins (B-complex and vit. C) and other bioactive compounds (phenolic compounds, anthocyanins, sterols, and carotenoid) [9] give them a high-added value mainly related to their functional and healthy properties [1–3,10,11]. However, this composition highly depends on the ripening stage, cultivar, growth region, and climatic conditions [12,13]. Through their ripeness stages (hababuk, kimri, khalal, rutab and tamar), date fruits undergo relevant variations in the color, texture, taste, and chemical composition [14]. At the final stage of ripening (tamar), the fruit is completely ripened, and the moisture is also reduced, which is the best moment for its consumption.

From a commercial point of view, the Medjoul cultivar is the dominant one in the palm grove of Elche, leaving the autochthonous cultivars at a disadvantage both commercially and scientifically. The present study will provide scientific data that will give added value to the autochthonous Confitera cv., which is totally adapted to the edaphoclimatic conditions of this special growing area. The objective of this work was to compare fresh dates from Medjoul and Confitera cv. of the Elche palm grove at the tamar stage, by means of their morphological characteristics, physicochemical properties, nutritional composition, bioactive compounds content and antioxidant properties. These data will contribute to a better understanding of their overall quality and, to support decisions aimed at organizing the production of fresh dates from Elche by selecting the most profitable cultivars.

2. Materials and Methods

2.1. Raw Materials

Date fruits of cv. Medjoul and Confitera were harvested (manually picked, from October 2021–February 2022) at the tamar stage from date palm trees (7000 from each cultivar) located in the Elche palm grove (Elche, Alicante, Spain) by specialized staff and transported under refrigerated conditions to the IPOA laboratory of the Orihuela Campus at the Miguel Hernández University (Orihuela, Alicante, Spain). The average sample used in this work was 45 kg from each cultivar.

2.2. Morphological Characteristics

Morphological parameters consisting of date fruit weight, length, and width were measured from 20 healthy and uniform date fruits from each cultivar (Medjoul and Confitera) that were randomly selected. After that, each of the selected dates was manually peeled and the seed was separated; then, the weights of skin, pulp and seed were recorded. The fresh weights of the date, seed, and pulp were determined using an analytical balance

PLI-360-3M (Kern & Sohn, Balingen, Germany), and the length and width determined using a Vernier caliper. Mean values and standard deviations were recorded.

2.3. Physicochemical Properties

The pH of date fruit was determined using a calibrated pH-meter GLP21 (Crison, Barcelona, Spain) on a water suspension (0.5 g sample with 50 mL deionized water blended for 2 min).

The external color of date fruit was measured using a CM-700 Minolta spectrophotocolorimeter (Minolta, Osaka, Japan) in CIELAB mode under CIE Standard Illuminant D65 and observation angle 10°. CIELAB coordinates (lightness (L*), red/green (a*) and yellow/blue (b*)) were obtained from which the psychophysical magnitudes Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and Hue ($H^*$ = arctg b*/a*) were calculated. Color differences ($\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ between Medjoul and Confitera dates were also calculated.

2.4. Technofunctional Properties

The water-holding capacity (WHC), oil-holding capacity (OHC) and swelling capacity (SWC) of date fruit samples were measured following methods described by López-Marcos et al. [15]. To determine the WHC of the date fruit samples, a centrifuge tube containing 500 mg of date fruit was supplemented with 10 mL of ultrapure water. The tubes were then stored at 25 °C for 18 h. Subsequently, centrifugation was carried out using a centrifuge (Nahita 2652, Alicante, Spain) at 3000 rpm for 20 min at the same temperature. Following centrifugation, the supernatant was discarded, and the remaining pellet was weighed. The WHC value for each sample was calculated as the weight of water retained by 1 g of the corresponding date fruit sample. For OHC, a similar procedure previously described for WHC was applied but using 5 g of sunflower oil with 500 mg of date fruit samples, with the results expressed as the weight of oil held by 1 g of corresponding date fruit samples. For the SWC, date fruit samples (200 mg) were weighed in a 10 mL tube and the volume occupied by the date fruit sample was recorded before adding 5 mL ultrapure water. The mixture was stirred to eliminate any trapped air bubbles and then left on a level surface at room temperature for 24 h, allowing the sample to settle. The volume (mL) occupied by the hydrated date fruit samples was measured, and SC was expressed as milliliters per gram of date fruit samples.

2.5. Proximate Composition

Total ash (AOAC 923.03), protein (AOAC 981.10), fat (AOAC 991.36), dietary fiber (AOAC 985.29) and moisture content (AOAC 925.45) of date fruits were determined (in triplicate) using AOAC methods [16]. Total sugars content was estimated by difference, subtracting the sum of the other components (moisture, fat, protein, ash and TDF) from the total (100%).

2.6. Organic Acids and Sugars Profile

2.6.1. Extraction of Organic Acid and Sugars

Fifty mL of ultrapure water, acidified with *ortho*-phosphoric acid (0.1% v/v), were added to 1 g of the Confitera and Medjoul date fruit paste and stirred at room temperature for 24 h. Then, solutions were homogenized at 20,000 rpm for 2 min in a homogenizer (Ultra-Turrax T25 BASIC, IKA-Werke GmbH & Co. KG, Staufen, Germany) and heated at 80 °C for 1 h under constant stirring. After that, the samples were centrifuged at 6500× g for 10 min at 4 °C and the supernatants were filtered through a 0.45 µm filter.

2.6.2. HPLC Analysis

Organic acids and sugars were quantified according to Melgarejo-Sánchez et al. [17]. The HPLC system used was a Hewlett-Packard 1100 series model (Woldbronn, Germany). The samples (20 µL) were injected in a Supelco column (Supelcogel TM C-610H column 300 mm × 78 mm) and absorbance was measured at 210 nm using a diode-array detector

(DAD G-1315A). The elution buffer was *ortho*-phosphoric acid in water (0.1% *v*/*v*) with an isocratic flow rate of 0.5 mL/min. These same HPLC conditions (elution buffer, flow rate and column) were employed for the determination of sugars. Though, the detection was carried out by means of a refractive index detector (RID G1362A). Standards of organic acids, monosaccharides and oligosaccharides were obtained from Supelco (Sigma-Aldrich, St. Louis, MO, USA). Peaks were identified by comparison with theretention time of the standards, and quantified by regression formula obtained with the standards.

2.7. Mineral Composition

The determination and quantification of mineral content of the lyophilized date fruits samples (Freeze dryer Alpha 2–4, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) were carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS-2030) (Shimadzu, Kyoto, Japan). Mineral content was quantified after digestion with 67% nitric acid and 33% hydrogen peroxide by a microwave system. In order to estimate the content of the concentrations of macro- and micro-elements, calibration standards were prepared. The final value per sample was the average of 3 reads, and mineral content was expressed as mg/100 g dry weight of date fruit.

2.8. Polyphenol Composition

The extraction of polyphenolic compounds was conducted following the methodology described by Genskowsky et al. [18]. To minimize any potential interference from the sugar content in the samples during chromatographic analyses, a C-18 Sep-Pak cartridge was utilized. Prior to loading the extracts onto the cartridge, it was conditioned with 3 mL of methanol, 3 mL of ultrapure water, and 3 mL of hydrochloric acid (10 mM). Subsequently, the cartridge was washed with 5 mL of ultrapure water. The final step involved eluting the cartridge with 3 mL of acidified methanol (0.1 g/L formic acid). The resulting extracts were carefully preserved at −40 °C until HPLC analysis.2.8.2. HPLC analysis.

For the determination of polyphenolic compounds, a LC-MS/MS 8050 High Performance Liquid Chromatography triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used. The ISE source was operated with a nebulizer gas flow of 3 L/min, drying gas flow of 10 L/min, a desolvation line (DL) temperature of 250 °C, and a heat block temperature of 400 °C. Selected ion monitoring (SIM) with a collision energy of −35 V and full MS scans in positive mode between 100–1000 m/z. The chromatographic separations were performed with a Mediterranean SEA18 column (10 mm L × 0.21 mm i.d., 2.2 µm particle size, Teknokroma, Barcelona, Spain) maintained with a temperature at 50 °C. The mobile phase A consisted of 0.1% formic acid in ultrapure water and the mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient elution was 0–2 min 5% B, 2–10 min 95% B, 10–11 min 95% B, 11–12 min 5% B, and 12–16 min 5% B with a flow rate of 0.400 mL/min and injection volume of 10 µL. External standards were used for the quantification of polyphenolic compounds; a stock of 200 mg/L was made and from it a calibration plot of concentrations 0.1, 0.3, 0.5, 0.8, and 1 mg/L was obtained. Labsolutions LCMS software Ver. 5.98 (Shimadzu) was used for instrument control and data-processing. All analyses were done in triplicate.

2.9. Antioxidant Properties

The antioxidant properties of date fruits were assessed usingfourdifferent antioxidant assays. DPPH assay was performed following the method proposed by Brand-Williams et al. [19] TEAC-ABTS assay was established following the method proposed by Gullón et al. [20]. Ferric reducing antioxidant power (FRAP) was assessed by means of potassium ferricyanide-ferric chloride method described by Oyaizu [21]. Ferrous ions chelating activity (FIC) was determined establishing the inhibition of Fe^{2+} -ferrozine complex formation after adding to test material Fe^{2+} by means of the method described by Mahdavi et al. [22]. Absorbance values were measured on a spectrophotometer at 517 nm (for DPPH assay), 734 nm (for TEAC-ABTS assay), 700 nm (for FRAP assay) and 562 nm (for

FIC assay). Trolox was used as the reference standard for DPPH, TEAC-ABTS and FRAP assays, and EDTA for FIC assay. The results were expressed as μg Trolox Equivalents/g of date fruit in the case of DPPH assay, and as mg Trolox Equivalents/g of date fruit in the case of ABTS and FRAP assays. For the FIC assay, the results were expressed as μg Ethylenediaminetetraacetic Equivalents (EDTAE)/g of date fruit.

2.10. Statistical Analysis

All determinations were made in triplicate and results are shown as mean ± standard deviation. Comparison was conducted using the one-way Analysis of Variance (ANOVA) at a confidence level of 95%. These analyses were carried out with the statistical program SPSS for Windows v. 27.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussions

3.1. Morphological Characteristics

Figure 1 shows some date fruits atthe tamar stage of both cultivars, Confitera and Medjoul, while their morphological characteristics are shown in Table 1.



Figure 1. Date fruits at the tamar stage of Medjoul (**A**) and Confitera (**B**) cultivars.

Table 1. Morphological characteristics of Spanish-grown Medjoul and Confitera date fruits.

Date cv.	Length (cm)	Width (cm)	Total Weight (g)	Pulp Weight (g)	Skin Weight (g)	Seed Weight (g)
Medjoul	3.88 ± 0.36	2.14 ± 0.25	11.64 ± 3.85	9.08 ± 3.16	1.15 ± 0.37	1.23 ± 0.45
Confitera	4.58 ± 0.22	2.14 ± 0.17	13.12 ± 1.61	10.97 ± 1.48	1.09 ± 0.20	0.97 ± 0.17
p value	0.000	0.943	0.122	0.021	0.485	0.018

These results indicate that the dates of the Confitera cv. are longer ($p < 0.05$) than those of Medjoul. Although there are no differences in the total weight of the dates of both cultivars, the Confitera date fruits have a higher proportion ($p < 0.05$) of pulp than seed in comparison with Medjoul date fruits, which results in a higher pulp yield (84% for Confitera cv. vs. 78% for Medjoul cv.), since the edible part is the pulp being the seed considered a coproduct. The morphological characteristics of Medjoul dates are within the range of the data reported for this cultivar in different growing areas [8,23,24]. No bibliographic references have been found on the morphological characteristics of Confitera dates at the tamar stage. It is also important to note that these properties are highly dependent on the ripening stage, and it is not during the tamar stage (fully ripe stage) that the highest values (weigh, size and yield) are reached [24–26]. These authors reported that date fruits grew considerably until the khalal stage, mainly due to faster cell division and elongation processes, and then, from the halal to tamar stage a decreasing trend was observed, due to fruit shrinkage after reaching the final tamar stage. According to the quality standards of date fruits applied in the international scale reported by Meligi and Sourial [27], in

reference to the length, width, total weight, pulp weight and seed weight/date weight (%), all date fruits would receive the highest evaluation (good character), except Medjoul dates for the length that would be evaluated as medium quality (3.5–4 cm; acceptable).

3.2. Physicochemical Properties

The pH is one of the most important parameters affecting their processing and storage quality. The results shown in Table 2 reveal higher pH values ($p < 0.05$) in Confitera than in Medjoul dates. Both pH values are within the range reported for fruit dates at the tamar stage (5.2–6.3) [28,29]. In both cases, pH values were higher than 5, and the limit value associated with an acidic taste, evaluated as bad character by the consumer. The acidic nature of date fruits is due to the natural organic acids originating from date fruit including succinic, malic, tartaric and ascorbic acid (see Section 3.5).

Table 2. Physicochemical properties [lightness (L*), red/green coordinate (a*), yellow/blue coordinate (b*), chroma (C*) and hue (H*)] of Spanish-grown Medjoul and Confitera date fruits.

Date cv.	pH	L*	a*	b*	C*	H*
Medjoul	5.74 ± 0.02	32.50 ± 1.11	4.39 ± 0.65	4.96 ± 1.04	6.63 ± 1.20	48.22 ± 2.04
Confitera	5.94 ± 0.04	32.36 ± 0.30	5.13 ± 0.35	5.85 ± 0.33	7.78 ± 0.36	48.72 ± 2.32
p value	0.003	0.732	0.009	0.028	0.014	0.640

Color plays a key role in the quality index and marketing value of fruits. In the case of date fruits, intense color variations are closely related with cultivar and ripening progresses [14,30,31]. Nevertheless, different date cultivars exhibit their own color upon ripening [31]. During ripening, evident changes in the color of date fruits results from the degradation of chlorophyll, marking the transition from one stage to another. Date fruits are rich in carotenes, orange-yellow to red crystalline pigments (fat-soluble) which are responsible for their bright and typical color. Furthermore, it has been reported that during ripening the concentration of chlorophyll decreases but the carotenoid concentration does not improve or even decrease, which is represented by the presence of yellow-brown color in dates [14,32]. At the last ripening stage (tamar), date fruits are usually less luminous and darker than at the first stages, which is related to the loss of water [33]. Color parameters of the surface of Medjoul and Confitera date fruits at the tamar stage are shown in Table 2. Regarding color coordinates, Confitera dates showed higher a* and b* values ($p < 0.05$) and a similar lightness ($p > 0.05$) than Medjoul dates. In any case, as the color differences (ΔE*) were lower than three units (1.16 ± 0.02), they cannot been detected by the human eye. Confitera dates showed a more saturated color ($p < 0.05$) than Medjoul dates although the hue of the dates from both cultivars was the same ($p > 0.05$), corresponding to a red-orange hue [34]. In addition to the content and/or proportions of natural pigments (chlorophylls, carotenes, anthocyanins, etc.), the color of dates is also influenced by the development of non-enzymatic browning reactions (Maillard reaction and caramelization) promoted by their high content of reducing sugars (see Section 3.5).

3.3. Techno-Fucntional Properties

Techno-functional properties (WHC, SWC and OHC) of Confitera and Medjoul date fruits are shown in Figure 2.

The hydration properties (WHC and SWC) of food materials are important not only for its physiological role but also for their influence on the techno-functional properties of the food. WHC and SWC provide information regarding the hydration capacity of the food matrix and give insights into its behavior during gut transit and food processing [15,35]. It has been reported that food matrices with good hydration properties (high WHC and SWC) could be added as a functional ingredient in the development of functional foods due to their physiological effect during digestion, absorbing water in the gut contributing to stool bulking [36,37]. However, other authors have reported that a high affinity to water could show some negative effect on the texture and shelf life of the final food [36,38].

Medjoul and Confitera dates showed similar ($p > 0.05$) hydration properties (WHC and SWC), being SWC values higher ($p < 0.05$) than corresponding WHC values. It must be noted that the WHC is the ability of a material to retain water when subjected to an external force (centrifugation or pressure), so it consists of the sum of bound water, hydrodynamic water and, mainly, physically trapped water. However, the SWC represents the amount of water that can be absorbed, and it is regarded as an indicator of a structure's aptitude to spontaneously absorb water when in contact with a constantly moist surface or when immersed in water. For this reason, it is expected that SWC showed higher values than WHC. In any case, it could be said that Medjoul and Confitera date fruits at the tamar stage have no good hydration properties, in comparison with other vegetable matrices [39,40]. No data have been found on the hydration properties of date pulp as such, but there are much data referring to extracts rich in dietary fiber obtained from the date pulp of several cultivars [29,41,42] and even from the bagasse obtained after the extraction of date juice [43]. In both cases, the values reported are higher than those obtained here, but this is due to the high hydration properties attributed to dietary fiber, which in these extracts is in larger proportions than those present in fresh date pulp (raw material).

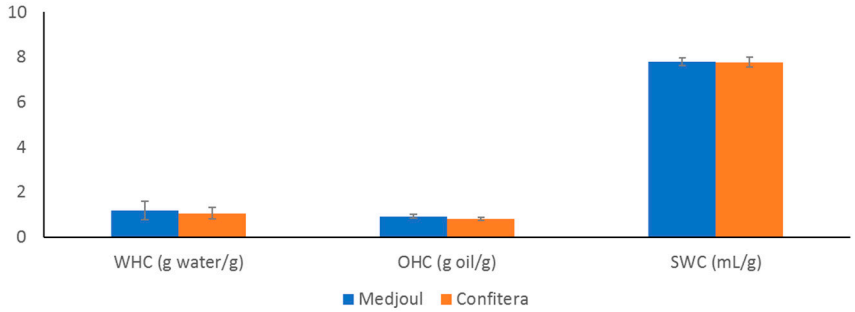


Figure 2. Techno-functional properties of Spanish-grown Medjoul and Confitera date fruits.

The OHC is also an important technological property that reflects the amount of oil retained in the matrix after incubation with oil and centrifugation and depends on the chemical and physical structure of polysaccharides, their surface properties and porosity [15,44]. Again, there were no differences between ($p > 0.05$) Medjoul and Confitera dates for OHC. Similar OHC values have been reported for some vegetable matrices such as passion fruit, pineapple, guava, or apples [40].

3.4. Proximate Composition

The results of the proximate composition of Confitera and Medjoul dates are shown in Table 3. Total sugars were the predominant fraction in date pulp from both date cultivars, followed by moisture and total dietary fiber, with small amounts of proteins, fats and ashes.

Table 3. Proximate composition (mg/100 g) of Spanish-grown Medjoul and Confitera date fruits.

Date cv.	Moisture	Protein	Fat	TDF [1]	Ash	Sugars
Medjoul	32.38 ± 0.52	1.98 ± 0.05	0.37 ± 0.05	20.05 ± 0.81	2.05 ± 0.07	43.17 ± 0.67
Confitera	25.65 ± 0.65	2.58 ± 0.18	0.18 ± 0.04	21.94 ± 0.94	2.06 ± 0.03	47.59 ± 0.76
p value	0.000	0.032	0.009	0.291	0.820	0.043

[1] TDF: Total Dietary Fiber.

Medjoul dates showed higher ($p < 0.05$) moisture content than Confitera. It is widely documented that the moisture content of date pulp decreases throughout the different stages of ripening, with the last stage (tamar) having the lowest moisture content [1]. Depending on cultivar and growing conditions (place, climate, etc.) large differences in the moisture content of date pulp have been reported, ranging from 9 to 32% [28,45], being our data within this range and close to the highest moisture content. Protein, fat, and ash occur in small amount in date fruits, observing that Confitera dates had lower ($p < 0.05$)

fat and higher ($p < 0.05$) protein content than Medjoul, but the same ash content ($p > 0.05$). Although the protein content in dates is not too relevant, they have been reported to contain high amount of some essential amino acids [3,12], some of which are not present in the most popular fruits, such as oranges, apples and bananas. It has been reported that the fat, protein and ash content decreases throughout the ripening process of the dates, reaching the lowest values at the end (tamar stage) [46]. On the contrary, simple carbohydrates content increases through ripening [28]. Medjoul dates showed lower ($p < 0.05$) total sugar content than Confitera, being this content, in both cases (63.8 and 64.9 mg/100 g dw., respectively), is lower than the one reported for other cultivars at tamar stage (73–88 g/100 g dw) [29,45]. There were no significant differences ($p > 0.05$) in TDF content between Medjoul and Confitera dates, being these results are higher than those reported for dates of different cultivars and the degree of ripeness (6.4–15.7 g/100 g) [47,48]. As well as total sugar concentration, TDF content is also dependent on the date cultivar, ripening stage, and climatic and growing conditions [47,49,50]. On the contrary to that reported for total sugars, TDF content decreases with the fruit transition from kimri to tamar stages, which has been attributed to the gradual enzymatic breakdown of these substances to more soluble compounds that soften the fruit [3,45,50]. In any case, even at the tamar stage, date pulp can be considered a good source of TDF (with higher IDF content than SDF) [1,29].

3.5. Sugars and Organic Acids

While sugars are responsible for the sweetness of date fruits, organic acids are responsible for their taste, contributing to sourness and modulating their sweetness. The content of the sugars and organic acids of Confitera and Medjoul date fruits is shown in Figures 3 and 4, respectively.

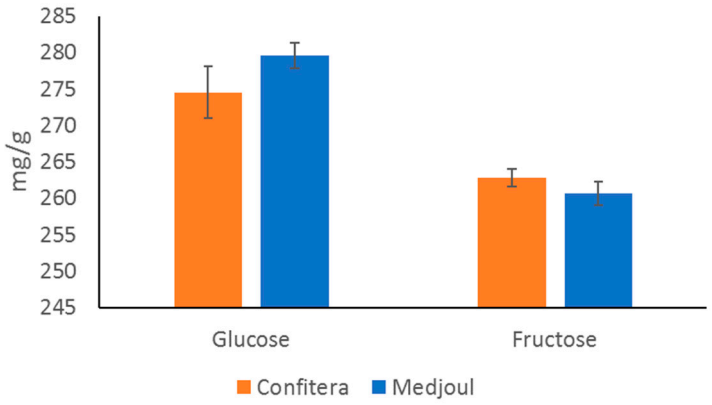


Figure 3. Sugar content (mg/g) of Spanish-grown Medjoul and Confitera date fruits.

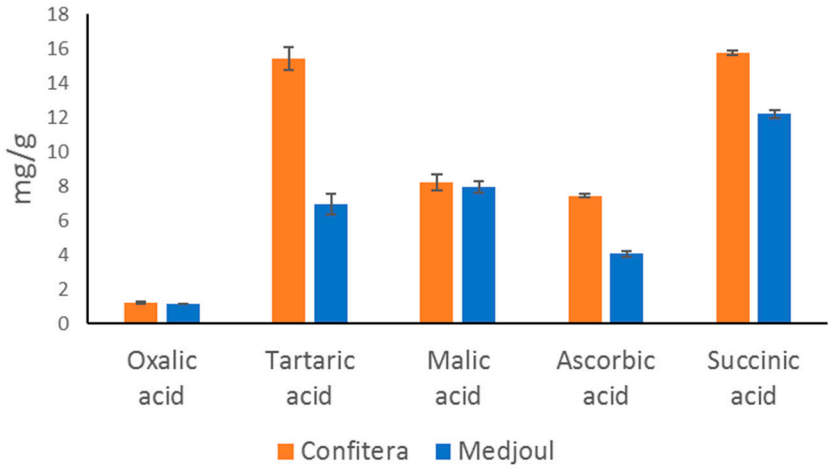


Figure 4. Organic acid content (mg/g) of Spanish-grown Medjoul and Confitera date fruits.

Date pulp is characterized by its high sugar content, with the major sugars being glucose (the highest amount in Confitera dates, $p < 0.05$) and fructose (with no differences between cultivars, $p > 0.05$) in a similar proportion. Similar values of reducing sugars have been reported for Medjoul dates grown in Mexico [8]. These two reducing sugars have also been reported as the main sugars in several date cultivars (Alligh, Boufeggous, Goundi, Ikhouat, Kenta, Lagou, Touzerzaillet, Ajwa, and Tranja, among others) at the tamar stage [29]. It is important to compare sugar content among cultivars but at the same ripening stage because it has been reported a relevant increase of sugars during ripening [29,48,49], being that the sugars are at the highest concentration during the tamar stage. Alternatively, other authors have reported higher amounts of sucrose than reducing sugars in several varieties (Sokari, Mabroom and Deglet Noor), but also in this case, their proportion was depending on ripening stage. They reported that sucrose was present only at the khalal and rutab stages (and not at the tamar stage) [49], in agreement with our results. The reason given by these authors to explain the sudden drop in sucrose and the increase in reducing sugars at the tamar stage was the rising activity of the invertase enzyme implied in the hydrolysis of sucrose into fructose and glucose. Regarding this, it could be said that these two sugars are responsible for the sweetness of the fruit and also for their softness (along with moisture) and they would even contribute to fruit color through Maillard and caramelization reactions [31].

Four major organic acids were detected in Confitera and Medjoul date fruits, mainly succinic, tartaric, malic and ascorbic acid with a smaller concentration of oxalic acid. Confitera dates showed higher ($p < 0.05$) amounts of tartaric, succinic and ascorbic acid than Medjoul dates and similar content of oxalic and malic acids ($p > 0.05$). All these organic acids have been found in dates from several cultivars, ripening stages, and growth conditions although with differences in their proportion [31,39,48]. In general, it could be said that total acidity should decrease during ripening, but with significant changes in the predominance of specific organic acids [31]. In addition to their effect on date taste, organic acids are also important for date quality due to their potential effects as preservatives (antimicrobial agents).

3.6. Mineral Composition

Although it has been reported that mineral content in date fruits decreases during ripening [28,51], even at the last ripening stage (tamar), dates can be considered, nutritionally, a significant source of important minerals in the diet. The mineral content of Medjoul and Confitera dates is shown in Table 4. The most abundant mineral found in both date fruits were potassium, followed in descending order by calcium or magnesium (depending on the cultivar; calcium in Medjoul vs. magnesium in Confitera), sodium, iron, zinc, copper and manganese. The same order has been reported by other authors in date fruits of different cultivars and growing places [8,29,51,52] confirming that date fruits are rich in most of the macroelements but poor in microelements. Confitera dates showed higher amounts ($p < 0.05$) of iron, potassium, magnesium, manganese, sodium and zinc than Medjoul dates and similar amounts ($p < 0.05$) of calcium and copper. What is interesting is the low sodium:potassium ratio of both cultivars, which is in line with the current dietary recommendations to decrease the risk of cardiovascular diseases [53]. Comparing the mineral content of these Medjoul dates with data from the same cultivar but growing in other countries, several differences have been noted: Spanish dates showed lower content in potassium than Medjoul dates from Mexico or Morocco (851 and 849 mg/100 g dw, respectively) [8,52], but higher amount of magnesium, calcium, sodium, zinc and copper than Moroccan dates (68, 54, 11, 0.37 and 0.34 mg/100 g dw, respectively) [52].

Table 4. Mineral content (mg/100 g dry weight) of Spanish-grown Medjoul and Confitera date fruits.

Component	Medjoul	Confitera	*p* Value
K	639.39 ± 47.67	837.33 ± 68.82	0.013
Mg	89.82 ± 5.37	114.65 ± 7.41	0.009
Ca	100.62 ± 5.50	98.65 ± 1.72	0.325
Na	30.68 ± 0.61	43.15 ± 0.85	0.031
Fe	1.20 ± 0.01	1.70 ± 0.30	0.024
Zn	0.83 ± 0.08	0.99 ± 0.09	0.019
Cu	0.65 ± 0.07	0.62 ± 0.03	0.287
Mn	0.39 ± 0.02	0.50 ± 0.01	0.037

3.7. Phenolic Composition and Antioxidant Activity

Date fruits can be considered a good source of antioxidant compounds, mainly polyphenolic compounds, especially in the earlier edible ripening stages. The nature, formulation, and distribution in dates vary with different factors such as variety, ripening stage, location, and environmental conditions [1,12,13,54]. The phenolic composition of Medjoul and Confitera date fruits growing in Spain is shown in Table 5. Eight polyphenolic compounds (seven flavonoids and one phenolic acid) were identified in Confitera dates and only six (five flavonoids and one phenolic acid) in Medjoul dates.

Table 5. Polyphenol composition (µg/g) of Spanish-grown Medjool and Confitera date fruits.

Compound	Medjoul	Confitera	*p* Value
(−) Epicatechin	LOD	1241.90 ± 1.13	-
Luteolin-7-O-glucoside	26.61 ± 0.74	8.21 ± 0.47	0.012
(+) Catechin	LOD	1150.89 ± 1.86	-
Caffeic acid	3.37 ± 0.09	3.32 ± 0.06	0.231
Eriocitrin	8.76 ± 0.23	10.85 ± 0.12	0.027
Hesperidin	7.47 ± 0.32	16.76 ± 0.41	0.013
Rutin	11.00 ± 0.48	12.97 ± 0.63	0.042
Isoquercitrin	19.03 ± 0.74	10.43 ± 0.36	0.031

LOD: lower than limit of detection.

Both flavonoids and phenolic acids have been reported as the main polyphenol groups found in date fruits [55]. More than 13 different flavonoid glycosides and 19 isomeric forms of flavonoids have been identified in date fruits by several authors [12,13,49,56]. In addition, cinnamic acid derivatives are usually found in date fruits from several varieties [57,58]. Tassoult et al. [49] analyzed the phenolic profile of several date varieties from Algeria, reporting that although compound patterns exhibited significant variations based on the cultivars, solvent used, and the stage of ripening, caffeic acid was the major phenolic acid, and catechin and luteolin the major flavonoids found in all samples. Confitera dates presented higher amount of epicatechin, catechin, eriocitrin, hesperidin and rutin, but a lower amount of luteolin-7-O-glucoside and isoquercitrin, than Medjoul dates ($p < 0.05$). There were no significant differences ($p > 0.05$) in the content of caffeic acid between both date fruits. Caffeic acid has been reported as one of the major phenolic compounds found in dates of different varieties and locations [10,59,60]. As has been previously commented, each cultivar of date synthesizes characteristic flavonoids that, depending on different environmental conditions (temperature, hours of light), sanitary conditions or growing conditions, expressed in higher or lower concentrations, which could explain the differences found in polyphenol composition between Medjoul and Confitera dates.

The total antioxidant activity of Confitera and Medjoul dates assessed by means of four different methods (DPPH, ABTS, FRAP and FIC) is shown in Table 6.

Table 6. Antioxidant properties of Spanish-grown Medjoul and Confitera date fruits.

Date cv.	FRAP (mg TE/g Pulp)	DPPH (μg TE/g Pulp)	FIC (μg EDTA/g Pulp)	ABTS (mg TE/g Pulp)
Medjoul	0.64 ± 0.05	26.46 ± 2.11	3.75 ± 0.39	1.14 ± 0.28
Confitera	0.78 ± 0.06	32.33 ± 3.13	4.63 ± 0.87	0.91 ± 0.14
p value	0.001	0.003	0.046	0.097

TE: Trolox Equivalents.

These methods have been trying to cover the different mechanisms implied in the antioxidant activity (scavenging activity, reducing power and metal chelating). Confitera dates showed higher ($p < 0.05$) antioxidant values than Medjoul dates for the DPPH, FRAP and FIC methods and similar ($p > 0.05$) values for the ABTS method. Several authors have reported good antioxidant activity of date fruits of several cultivars (Tantebouchte, Biraya, Degla Baidha, Deglet-Nour, Ali Ourached, Ghars, and Medjoul, among others) obtained from different countries (Algeria, Kuwait Oman, Iran, Saudi Arabian, USA, Mexico, etc.) [2,49,60,61]. Allaith [62] reported similar values for the antioxidant activity, using the FRAP assay, of Bahrain dates at the tamar stage. Most of these authors confirmed that the antioxidant potential of date fruits was significantly correlated with the total phenolic and flavonoid compounds present in these fruits. In this case, a similar trend can be observed because Confitera dates showed higher total antioxidant activity than Medjoul dates, corresponding with their higher content in polyphenolic compounds, mainly flavonoids. In any case, it cannot be forgotten that other compounds found in date pulp (anthocyanins, phytosterols, carotenoids, and selenium) could also contribute to its antioxidant properties [1,2,63]. In agreement with data previously reported, Spain-grown Confitera and Medjoul dates contain good amounts of different polyphenolic compounds which have shown in vitro antioxidant potential, making them not only a valuable food, but also a promising ingredient in the development of functional foods.

4. Conclusions

Based on the results, it could be said that the growth and production of date fruits of the autochthonous Confitera cultivar should be promoted in this region (Southeast of Spain: Elche palm grove) not only for their contribution to sustainable agricultural development and biodiversity, but also for their higher overall quality in comparison with date fruits from Medjoul cultivar growth in similar conditions. Confitera dates are longer and have higher pulp yield than Medjoul. Nutritionally, both dates are a good source of carbohydrates and dietary fibers, with a small amount of fats and proteins. The main free sugars in dates from both cultivars were glucose and fructose (reducing sugars). The most abundant minerals found in both date fruits were K, followed by Ca or Mg (depending on the cultivar; Ca in Medjoul and Mg in Confitera). In addition, although both cultivars are rich in polyphenols, mainly flavonoids, with remarkable in vitro antioxidant potential, Confitera dates show a higher number of polyphenol compounds (and at higher concentrations) than Medjoul dates. In general, it can be said that fresh Confitera and Medjoul dates from the Southest of Spain can be considered valuable foods and promising ingredients in the development of functional foods.

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7.2. PUBLICACIÓN 2

Development of value-added products suitable for food applications from fresh date fruit (Confitera cv.) and its co-products

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RESEARCH

Development of Value-Added Products Suitable for Food Applications from Fresh Date Fruit (*Confitera cv.*) and its Co-products

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Abstract

The increasing commercialization of fresh date fruits (Confitera cv) in Spain is generating important amounts of co-products which currently are discarded as waste with the corresponding environmental problem and economic losses. The aim of this work was to valorize them, in an integral way, applying non-polluting procedures (grinding, soaking, filtering, or drying) allowing their reincorporation in the food chain in function on both nutritional and technological properties. Different intermediate and stable products with high added value have been obtained: (1) Date pastes with 50% moisture content and the same amount of sugars and dietary fiber (20% approx.), good source of K, Ca, and Mg, with low Na/K ratio,whose technological properties give them a great potential to provide desirable texture properties in some foods; (2) date waters rich in sugars and minerals with potential application as natural sweeteners or as source of carbon for the microbiota in fermented foods; (3) date flours with low moisture and high TDF content (58–66%), rich in minerals, and whose technological properties allow them to be used as carrier of oils (i.e., with healthy lipid profile) or as an emulsion stabilizer in the development of new foods.

Keywords Date pastes · Date flour · Date water · Valorization

Introduction

In the world, over 9 million tons of date fruits are annually produced (FAO, 2023) and about 30% are discarded or wasted due to low-grade classification (size, color, insects or natural damages, etc.) for their commercialization. These co-products could be subjected to several treatments for converting them, through low environmental impact procedure, into raw materials with added value for food applications (Echegaray et al., 2021; Muñoz-Tebar et al., 2023), returning to the food chain and thus contributing to the circular economy as well as fulfilling with the Sustainable Development Goals (FAO, 2018).

The growing interest in the valorization of agro-industrial co-products has led to the search for efficient treatments that also environmentally friendly. In this sense, several pretreatments and treatment processes (chemical, physical, and biological) have been applied to agro-industrial co-products depending on the original matrix and the amount of value-added compounds that they contain (Ahmed et al., 2021; Almaraz-Sánchez et al., 2022). For example, dietary fiber concentrates are the most reported value-added compounds extracted from fruit and vegetable co-products (Bchir et al., 2014; Borchani et al., 2012; García-Amezquita et al., 2018). However, in all these cases, the process used focuses on obtaining a single value-added product with a very specific characteristic (high dietary fiber content), undervaluing other compounds of high food value that are discarded during processing.

Date fruits have a valuable content of nutritional components (sugars, dietary fiber, certain essential vitamins, and minerals) and bioactive compounds (polyphenolic compounds, anthocyanins, sterols, and carotenoids) which can be recovered by applying the adequate process, transforming their co-products into new products, or extracting the compounds of interest (Echegaray et al., 2021; Muñoz-Tebar et al., 2023). This composition is highly related to the cultivar, growing place and conditions, and ripening

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stage of date fruits (Amira et al., 2012; Fernández-López et al., 2022; Hussain et al., 2020). Through their ripening stages (hababuk, kimri, khalal, rutab, and tamar), date fruits undergo relevant variations not only in their composition but also in the color, texture, and taste (Al-Qarni & Bazzi, 2020; Eid et al., 2013). Therefore, the application of a process (by using simple and environmentally friendly operations) that allows the valorization of date co-products in an integral way, obtaining different value-added products which, due to their specific characteristics, could be applied as functional ingredients in the food industry, would represent a great advance in the reduction of food waste in line with the UN's sustainable development goals, and would contribute to the circular economy as well.

Confitera is an autochthonous cultivar of date palm from the southeast of Spain (Elche palm grove, UNESCO World Heritage Site) that is been promoting in this area as an alternative to other cultivars worldwide and widely distributed (Medjoul, Deglet Noir, Hayani, etc.). Confitera is a cultivar well adapted to the specific edaphoclimatic conditions in European oasis among that contributes to maintain local cultivars, Ilicitan ecosystems, and plant diversity (Martín-Sánchez et al., 2014; Pérez-Álvarez et al., 2023). Confitera dates are harvested (October–April) at the three last ripening stages: khalal (firm skin and hard texture, bright yellow color, with high sugar content but also strong tannins that can be overpowered), rutab (at this stage fresh dates are at their best, the hard skin begins to soften at the tip and turn to brown color, advancing while the fruit continue to ripen until a combination of sweet juiciness with a slightly crunchy texture is reached), and tamar (fully ripe dates, easy to peel, dark brown color, very sweet, and full of aromas and flavors) (Pérez-Álvarez et al., 2023).

The aim of this work was to valorize Confitera date co-products (at different ripening stages) applying efficient and green processes in order to obtain value-added products suitable for food applications. In addition, the whole characterization of all the value-added products obtained along with its processing has been assessed in view of selecting, for each product, the most suitable food application.

Materials and Methods

Raw Materials (Date Fruit Co-products)

Date fruits (Confitera cv.) from Elche palm grove (Alicante, Spain) at different ripening stages (khalal, rutab, and tamar) and with no commercial value (below standard sizes, with minor physical damage due to bruises or insects) were provided by the Catedra Palmeral d'Elx (UMH, Alicante, Spain).

Date Fruit Co-product Processing

Date fruit coproducts were processed applying only physical treatments such as milling, soaking, pressing, and drying (Fig. 1). Date co-products were separated in three batches depending on the ripening stage (khalal, rutab, and tamar) and then they were independently processed. Date seeds were manually separated, and the rest (date pulp and peel) was ground using a cutter 1094-Homogeneizer (Tekator, Höganäs, Sweden) to obtain date paste. This date paste was soaked with distilled water 3 times. For the first two soakings, the proportion of water added was 1:1 (paste:water), while in the third soaking it was necessary to increase the proportion of water to 1:2. After every soaking, the corresponding date waters were separated from the date paste by pressing, using cotton filter clothes. Then, the solid part was distributed on trays and dried in an oven with forced ventilation at 60 °C for 24 h. The dried date paste was milling and sieved to obtain the date flour with a particular size > 0.52 mm.

From the initial raw material (date co-products at each of the 3 ripening stages) five value-added products were obtained (date paste, 3 date waters, and date flour). So, 15 value-added products were obtained in total: KHDP, date paste at khalal stage; RTDP, date paste at rutab stage; TMDP, date paste at tamar stage; KHDW1, date water at khalal stage from soaking 1; RTDW1, date water at rutab stage from soaking 1; TMDW1, date water at tamar stage from soaking 1; KHDW2, date water at khalal stage from soaking 2; RTDW2, date water at rutab stage from soaking 2; TMDW2, date water at tamar stage from soaking 2; KHDW3, date water at khalal stage from soaking 3; RTDW3, date water at rutab stage from soaking 3; TMDW3, date water at tamar stage from soaking 3; KHDF, date flour at khalal stage; RTDF, date flour at rutab stage; TMDF, date flour at tamar stage.

Date Paste Analysis

The date pastes obtained from the processing of date coproducts at each ripening stages (KHDP, RTDP, and TMDP) were subjected to the following analysis.

Proximate Composition

Total moisture content (AOAC 925.45), protein (AOAC 981.10), fat (AOAC 991.36), ash (AOAC 923.03), and dietary fiber (AOAC 985.29) of date paste were assessed (in triplicate) following AOAC methods (AOAC, 2006). Total sugar content was calculated by difference, subtracting the

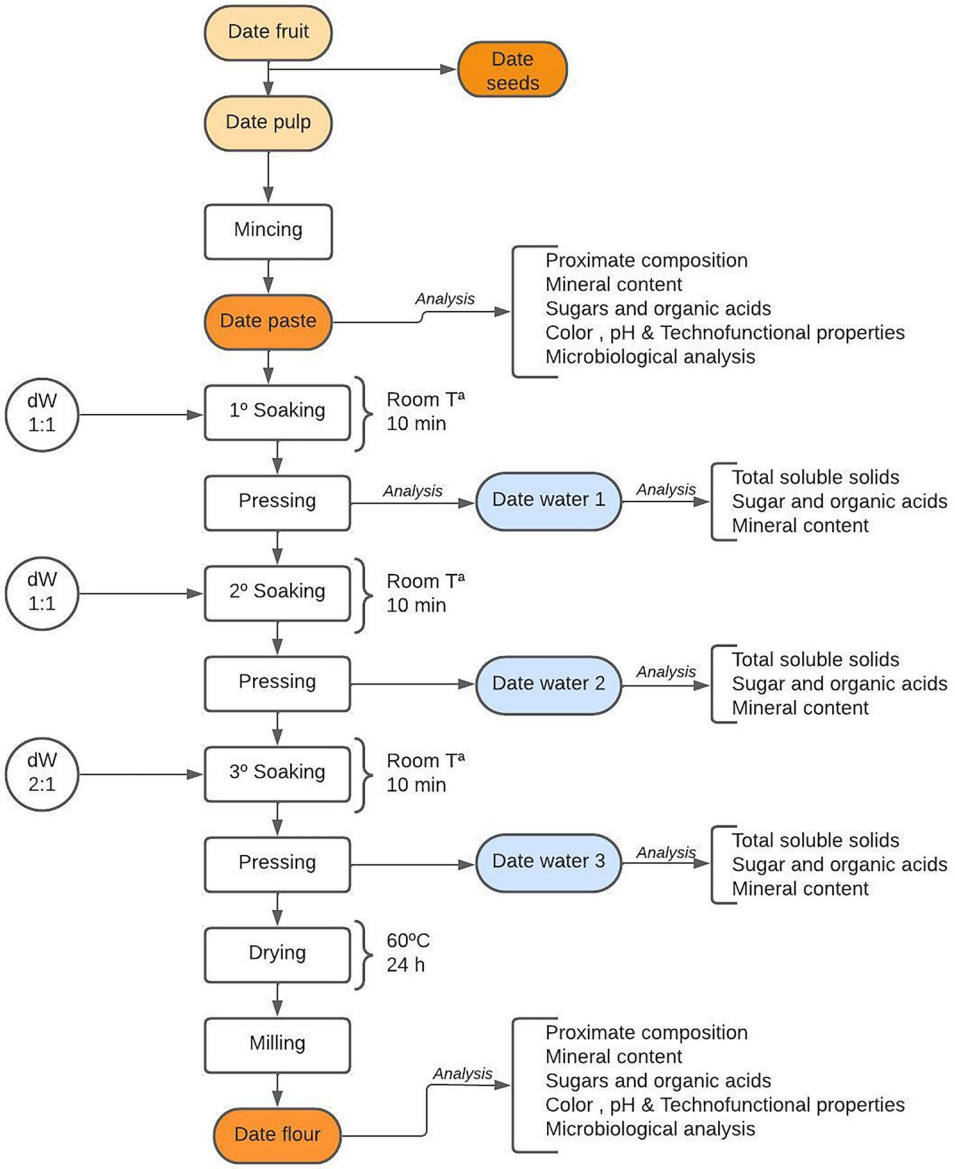


Fig. 1 Flow chart of the process for obtaining high value-added products from date co-products and the analyses applied for their characterization

sum of the other components (moisture, protein, fat, ash, and dietary fiber) from the total (100%).

Sugar and Organic Acid Profile

For the extraction of sugars and organic acids from date paste samples, 2 g of each date paste was mixed with 50 mL of ultrapure water and stirred at room temperature for 24 h. Then, these solutions were homogenized at 20,000 rpm for 2 min (Ultra-Turrax T25 BASIC, IKA-Werke GmbH & Co. KG, Staufen, Germany) and heated at 80 °C for 1 h, under constant stirring. After their centrifugation (6500×g for 10 min at 4 °C) the supernatant was filtered through a 0.45-µm filter.

Organic acids and sugars were quantified by HPLC analysis (Hewlett-Packard 1100 series model, Woldbronn, Germany) following the procedure described by Lucas-González et al. (2018). The samples (20 µL) were injected in a Supelco column (Supelcogel TM C-610H column 300 mm×78 mm) using as elution buffer ortho-phosphoric acid in water (0.1% v/v) with an isocratic flow rate of 0.5 mL/min. For organic acid determination, the absorbance was measured at 210 nm using a diode-array detector (DAD G-1315A), while sugar determination was carried out by means of a refractive index detector (RID G1362A). Peaks were identified by comparison with retention time of the standards (organic acids, monosaccharides, and oligosaccharides from Supelco, Sigma-Aldrich, St. Louis, MO, USA), and quantified by regression formula obtained with the standards.

Mineral Content

The mineral content was determined using inductively coupled plasma–mass spectrometry (ICP-MS) Shimadzu MS-2030 (Shimadzu, Kioto, Japan). The standard compounds were diluted and utilized to calibrate the ICP-MS for mineral analysis in date samples. ICP-MS operated with the follow conditions: nebulizer gas flow, 0.91 L/min; radio frequency 1200 W lens voltage 1.6 V; cool gas 12.0 L/min; auxiliary gas 0.70 L/min.

Physicochemical Properties

The pH was determined in a suspension with water (blending 1 g sample with 9 mL deionized water for 2 min) by using a pH-meter GLP21 (Crison Barcelona, Spain). The color of date pastes was measured using a CM-700 Minolta spectro-photocolorimeter (Minolta, Osaka, Japan) in CIELAB mode selecting the illuminant D65 and an observation angle 10°. CIELAB coordinates [lightness (L^*), red/green (a^*), and yellow/blue (b^*)] were obtained and the psychophysical magnitudes chroma [$C^* = (a^*2 + b^*2)1/2$] and hue ($H^* = \text{arctg}\ b^*/a^*$) were calculated from them.

Technofunctional Properties

The water-holding capacity (WHC), oil-holding capacity (OHC), and swelling capacity (SWC) of date pastes were evaluated according procedure described by López-Marcos et al. (2015). For WHC and OHC, 10 mL of ultrapure water or 5 g of sunflower oil, respectively, was added into centrifuge tubes with date paste (500 mg). Then, the tubes were stored at 25 °C for 18 h and centrifuged (1200 g, 20 min at 25 °C) (Nahita Model 2652, Alicante, Spain). After discarding the supernatant, the pellet was weighed and the results were expressed as the weight of water held (WHC) or oil held (OHC) by 1 g of date paste. For the SWC, 200 mg of date paste was weighed in a 10-mL graduated tube (0.1 mL graduations) and the volume occupied by the date fruit sample was noted. After that, 5 mL of ultrapure water was added, stirring the tubes to eliminate entrapped air bubbles and keeping at room temperature for 24 h to allow the sample to settle. The volume (mL) occupied by the hydrated sample was measured, and SC was expressed as mL per gram of date paste.

Microbiological Analysis

Total aerobic bacterial, molds, and yeast and enterobacteria of paste at the 3 ripening stages were analyzed. Ten grams of date paste was homogenized with 90 mL of sterile 0.1% (w/v) peptone water in a masticator during 60 s. Decimal dilutions were prepared and 1 mL was seeded in duplicate in Petrifilm plates (3M, Madrid, Spain). For total aerobic bacterial counts, samples were seeded on aerobic count plates and incubated at 37 °C for 48 h. For enterobacteria, paste samples were seeded on Enterobacteriaceae count plates and incubated at 37 °C for 24 h. Finally, molds and yeasts were counted on rapid yeast and mold count plates after incubation at 25 °C for 72 h. Plates ranging from 30 to 300 colony-forming units (CFU) were manually counted and the results were expressed as logarithm CFU/g paste.

Date Water Analysis

Each of the date waters obtained from the processing of date coproducts at each ripening stages by 3 consecutives soaking and pressing (KHDW1, RTDW1, TMDW1, KHDW2, RTDW2, TMDW2, KHDW3, RTDW3, and TMDW3) was subjected to the following analysis.

Total Soluble Solids

Total soluble solids (TSS) were determined using a digital refractometer Milwaukee MA 871 (Milwaukee electronics, Milwaukee, WI, USA) and expressed as °Brix.

Sugar and Organic Acid Content

The methodology used was the same as described for date pastes.

Mineral Content

The methodology used was the same as described for date pastes.

Date Flour Analysis

The date flours obtained from the processing of date coproducts at each of the three ripening stages (KHDF, RTDF, and TMDF) were subjected to the same analysis than reported for date pastes.

Statistical Analysis

Data from this paper were analyzed using SPSS software (IBM SPSS Statistics version 26). One-way analysis of variance ANOVA was applied using a confidence level of 95% and Tukey test was carried out to determine any significant difference ($p < 0.05$) between the 3 ripening stages of Confitera dates (khalal, rutab, and tamar). All data in this paper are means ± standard deviations of greater than or equal to three separate experiments.

Results and Discussions

Date Pastes

The results of the proximate composition of date pastes are shown in Table 1. Since they are obtained from fresh dates, the predominant fraction in the three date pastes was moisture followed by total carbohydrates (sugars and dietary fiber) with small amounts of fat, ash, and protein. Regarding differences in proximate composition due to the ripening stage, only moisture, TDF, and sugar content showed significant differences ($p < 0.05$). Moisture and TDF content of date pastes decreased, and sugar content increased ($p < 0.05$) as the ripening stage progressed, which has been widely documented (Al-Qarni & Bazzi, 2020; Fernández-López et al., 2022). The gradual enzymatic breakdown of long carbohydrates, identified as TDF, produces more soluble compounds (sugars) responsible for the sweetness and softness of ripe dates that can explain the changes in the proportion of TDF and sugars (Al-Farsi & Lee, 2008; Echegaray et al., 2021). The decrease in moisture through ripening is due to the transpiration and drying caused by environmental heat and moisture. All these values are within the range reported for fresh dates from different cultivars (Al-Farsi & Lee, 2008) although relevant differences depending on cultivar, growth conditions, and origin have been reported.

Mineral composition of date pastes affected by the ripening stage is shown in Table 1. There is not a clear trend between mineral content and ripening stage because there are some minerals whose content was not affected ($p > 0.05$) by the ripening stage (Mg, Mn, and Zn), while some of them increased (K, Fe, Ca, and Cu) and Na decreased ($p < 0.05$). Overall, the most abundant mineral in the three date pastes was K, followed (in decreasing order) by Ca and Mg (Ca in RTDP and TMDP and Mg in KHDP), Na, Fe, Cu, Mn, and Zn. Therefore, date pastes can be considered good sources of macroelements but poor in microelements, in accordance with what has been previously reported for fresh dates (Bouhlali et al., 2017; Salomón-Torres et al., 2019). For their potential application in food processing, it is very interesting to highlight the low Na:K ratio which is in line with the official recommendations aimed at reducing the sodium content of foods as a way to decrease the incidence of cardiovascular diseases among the population (WHO, 2020).

Date pastes are characterized by their high sugar content being glucose and fructose (reducing sugars) the major ones (Table 1). The amount and type of sugar change according to variety and ripening stage (Al-Qarni & Bazzi, 2020). The absence of sucrose in some cultivars has been explained by the environmental and genetic factors that may affect the qualitative and quantitative compositions of the sugar fraction by altering the activity of the enzymes involved in the synthesis and breakdown processes (Rastegar et al., 2012). The content of both reducing sugars in date pastes increased with the ripening stage of the dates ($p < 0.05$) reaching the highest content in TMDP, being glucose content higher than fructose ($p < 0.05$) in all the samples. Consequently, glucose to fructose ratios were > 1. These results are comparable with those published previously on different date varieties (Hamad et al., 2015). The content of reducing sugars depends on the cultivar and is closely related to texture and color (Ghnimi et al., 2018). Reducing sugars makes dates softer and would be responsible for the color changes of the pastes if subjected to thermal treatments due to their participation as substrates of the Maillard or caramelization reactions (Ghnimi et al., 2018).

Regarding organic acid content (Table 1), it could be said that total acidity (the sum of the content of each of the organic acids present in the sample) decreased during ripening ($p < 0.05$), but with significant changes in the

Table 1 Proximate composition and mineral, sugar, and organic acid profile of date pastes from dates at different ripening stages (kahlal, rutab, and tamar)

	KHDP	RTDP	TMDP
Proximate composition (g/100 g)			
Moisture	54.67 ± 0.42^{a}	51.09 ± 0.31^{b}	48.25 ± 0.33^{c}
Fat	0.25 ± 0.13^{a}	0.17 ± 0.01^{a}	0.39 ± 0.33^{a}
Protein	1.13 ± 0.08^{a}	1.11 ± 0.01^{a}	1.16 ± 0.01^{a}
Ash	0.62 ± 0.42^{a}	0.64 ± 0.09^{a}	0.48 ± 0.22^{a}
TDF	21.99 ± 0.23^{a}	18.87 ± 0.29^{b}	18.61 ± 0.23^{b}
Sugars	21.34 ± 0.26^{c}	28.12 ± 0.14^{b}	31.11 ± 0.22^{a}
Mineral composition (mg/100 g)			
Ca	121.25 ± 4.16^{c}	143.87 ± 4.51^{a}	135.64 ± 2.24^{b}
Cu	0.73 ± 0.04^{b}	0.69 ± 0.00^{b}	0.79 ± 0.02^{a}
Fe	1.21 ± 0.06^{b}	1.13 ± 0.01^{c}	1.32 ± 0.04^{a}
K	613.76 ± 11.06^{c}	655.21 ± 14.55^{b}	658.28 ± 4.72^{a}
Mg	130.96 ± 13.46^{a}	130.79 ± 1.6^{a}	127.55 ± 2.20^{a}
Mn	0.66 ± 0.05^{a}	0.61 ± 0.02^{a}	0.65 ± 0.03^{a}
Na	4.43 ± 0.22^{a}	1.43 ± 0.09^{c}	2.12 ± 0.17^{b}
Zn	0.59 ± 0.06^{a}	0.57 ± 0.01^{a}	0.54 ± 0.04^{a}
Sugars (mg/g)			
Glucose	112.41 ± 5.17^{c}	168.79 ± 0.48^{b}	177.81 ± 4.60^{a}
Fructose	105.00 ± 2.95^{c}	113.84 ± 5.53^{b}	137.72 ± 8.52^{a}
Organic acids (mg/g)			
Oxalic acid	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}
Citric acid	1.49 ± 0.02^{a}	0.27 ± 0.04^{b}	0.20 ± 0.03^{b}
Tartaric acid	5.03 ± 0.14^{a}	nd	0.62 ± 0.05^{b}
Malic acid	4.23 ± 0.06^{a}	4.76 ± 0.18^{a}	3.65 ± 0.23^{b}
Succinic acid	15.20 ± 0.06^{a}	15.72 ± 0.50^{a}	10.53 ± 0.63^{b}

For the same compound, $^{a-c}$different letters in the same row indicate significant differences between samples (ripening stages) ($p < 0.05$)

nd not detected, *KHDP* date paste at kahlal stage, *RTDP* date paste at rutab stage, *TMDP* date paste at tamar stage

predominance of specific organic acids, as has been previously reported (Famiani et al., 2015; Ghnimi et al., 2018). Tartaric, malic, and succinic acids were predominant in KHDP and TMDP ($p < 0.05$), while tartaric acid was not detected in RTDP. The content of specific organic acids depends on the cultivar, growth conditions, and ripening stage (Famiani et al., 2015; Martín-Sánchez et al., 2014; Sánchez-Zapata et al., 2011). The five organic acids identified in date pastes have been detected in date fruits of different cultivars and origins (Hamad et al., 2015). In the case of date pastes, the content of organic acids is very important due to their effect on pH reduction, their role as natural preservatives, and for their effect on date paste taste (acidity).

Physicochemical properties of date pastes are shown in Table 2. Date paste from dates at khalal stage (KHDP; the least ripe) showed the lowest pH values ($p < 0.05$) which could be related to its highest acidity (lower organic acids content, Table 1). Regarding color properties, lightness decreased through ripening, obtaining the highest L^* values in date pastes at khalal stage (KHDP) and the lowest ($p < 0.05$) at tamar (TMDP). So, it could be said that TMDP is darker than RTDP and KHDP. Several authors have reported a close relationship between the moisture of food and its lightness: the greater the amount of surface water, the greater the light reflection and therefore the greater the lightness (Vera Zambrano et al., 2019). In this case the values of L^* in date pastes are in agreement with their moisture content (Table 1). The behavior of the rest of color properties (a^*, b^*, C^*, and H^*) was the same: KHDP and RTDP showed the highest values (without differences between them) and TMDP the lowest ($p < 0.05$). Color is one of the most affected aspects by the fruit ripening process, and in the case of date fruits, these changes are more evident, being used for their classification into the different ripening stages (khalal, rutab, and tamar). Changes in a^* and b^* coordinates have been related to the chlorophyll degradation, the carotenes and anthocyanin content (from orange-yellow to red pigments), and also to some non-enzymatic browning reactions (mainly Maillard reaction) due to their high reducing sugar contents (Al-Qarni & Bazzi, 2020). As the date ripening stage progresses, the color saturation (C^*) of the date pastes decreased obtaining the lowest C^* values ($p < 0.05$) in TMDP. H^* values of date pastes evolved from yellowish-orange (KHDP) to orange (RTDP and TMDP) as fruit ripening progresses.

Table 2 Physicochemical properties of date pastes from dates at different ripening stages (kahlal, rutab, and tamar)

Parameter	KHDP	RTDP	TMDP
pH	6.46 ± 0.21^{b}	7.22 ± 0.01^{a}	7.16 ± 0.01^{a}
L^*	40.32 ± 0.11^{a}	34.85 ± 0.50^{b}	30.29 ± 0.73^{c}
a^*	4.35 ± 0.26^{a}	4.48 ± 0.20^{a}	2.51 ± 0.09^{b}
b^*	10.73 ± 0.61^{a}	9.99 ± 0.74^{a}	4.91 ± 0.18^{b}
C^*	11.58 ± 0.63^{a}	10.95 ± 0.75^{a}	5.51 ± 0.18^{b}
H^*	67.90 ± 1.13^{a}	65.79 ± 0.78^{a}	62.87 ± 1.11^{b}

For the same parameter, [a–c]different letters in the same row indicate significant differences between samples (ripening stages) ($p < 0.05$)

L^* lightness, a^* red/green coordinate, b^* yellow/blue coordinate, C^* chroma, H^* hue

KHDP date paste at kahlal stage, *RTDP* date paste at rutab stage, *TMDP* date paste at tamar stage

In the potential use of date pastes as ingredients for the development and innovation of functional foods, it is fundamental to know not only the technological behavior they would have when incorporated into a food matrix but also their physiological effect during the gut transit (for example, absorbing water contributing to stool bulking) (Sahni & Shere, 2017; Viuda-Martos et al., 2010). Therefore, properties such as water and oil holding capacity and swelling capacity (techno-functional properties) give interesting industrial information that would allow to optimize both formulation and food processes in view to achieve desirable results. Figure 2 shows the techno-functional properties of date pastes as affected by the ripening stage of date fruits. Regarding hydration properties (WHC and SWC), their behavior was different depending on the ripening stage; WHC decreased as the ripening progresses while SWC increased ($p < 0.05$). WHC is mainly related to the chemical and physical structures of the polysaccharides, which can hold water by absorption and adsorption phenomena, and to the sugar content because of their hygroscopic properties. Date pastes exhibited a WHC ranging from 0.91 to 1.5 g water/g paste (TMDP and KHDP, respectively) and these values agree with those reported by other authors for date pastes of different cultivars and ripening stages (Martín-Sánchez et al., 2014; Sánchez-Zapata et al., 2011). As expected, SWC was higher than WHC because WHC only measures the ability to retain water after applying an external force (centrifugation), while SWC measures the ability to absorb water when the sample is in contact with a constantly moist surface (López-Marcos et al., 2015). Based on these hydration properties, date pastes could provide desirable texture properties in some foods. On the other hand, there is not a clear relation between OHC and the ripening stage because the lowest values ($p < 0.05$) were obtained in RTDP and the highest in KHDP and TMDP (without significant differences between them). In any case, OHC values ranged from 0.44 to 1.03 g oil/g paste, which is in accordance with OHC values reported for other date pastes (Martín-Sánchez et al., 2014; Sánchez-Zapata et al., 2011). In this case, it could be said that date pastes do not have good capacity to bind oily components and this could mean that they can be potentially used for decreasing the greasy sensation in some fatty foods or to decrease oil retention during food frying.

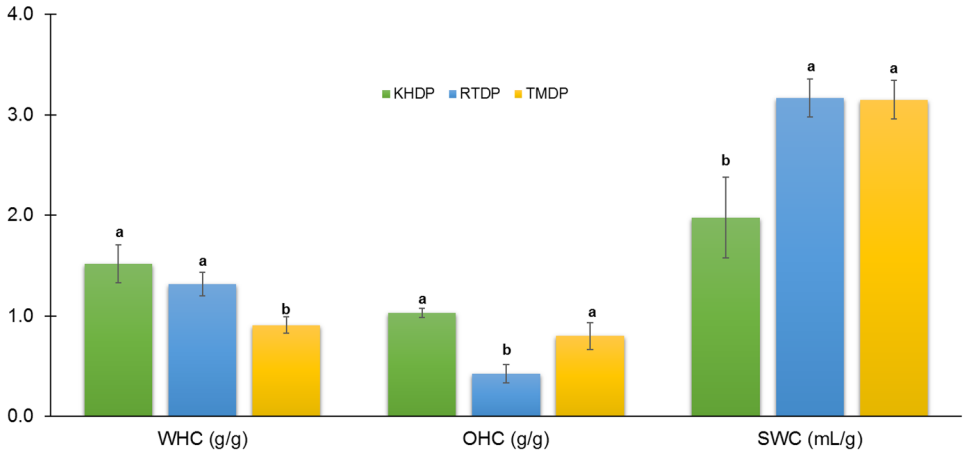


Fig. 2 Techno-functional properties (WHC, water holding capacity; OHC, oil holding capacity; SWC, swelling capacity) of date pastes from dates at different ripening stages (kahlal, rutab, and tamar). For the same parameter, (a,b) different letters indicate significant differences between samples ($p < 0.05$)

From a microbiological point, aerobic bacteria and enterobacteria were detected in all the samples, molds in KHDP and TMDP, and yeasts only in KHDP (Table 3). Regarding the ripening stage, there is not a clear trend in the microbiological quality. The European Union legislation regarding microbiological criteria for foods (Reglament CE 2073/2005), and particularly for fresh fruits minimally processed, does not include limits for this type of microorganism related to shelf-life decrease, only for pathogens (*Salmonell*a spp, and *Listeria monocytogenes*). However, the maximum allowed value for ready-to-eat foods is 10^5 CFU/g, as highlighted in the guidelines for microbiological quality (Gilbert et al., 2000). Counts > 10^6 CFU/g (10^8 CFU/g in some cases) would induce some visible modifications related to deterioration (Costa et al., 2019). On the present data, all date pastes showed microbial counts (for all microbial groups evaluated) lower than 10^4 CFU/g, meaning that they were stable during the study, in terms of refrigeration (4 °C). These microbiological counts are within the range of those reported by Nayik et al. (2013) and Ogodo et al. (2016) for fruit samples.

Table 3 Microbiological counts (log CFU/g) of date pastes from dates at different ripening stages (kahlal, rutab, and tamar)

Microorganism group	KHDP	RTDP	TMDP
Enterobacteria	1.04 ± 0.06^{a}	1.70 ± 0.01^{b}	0.39 ± 0.13^{c}
Molds	0.65 ± 0.07^{b}	nd	0.87 ± 0.04^{a}
Yeast	2.01 ± 0.02	nd	nd
Total aerobic bacteria	2.67 ± 0.01^{c}	3.12 ± 0.02^{a}	3.05 ± 0.02^{b}

For the same microorganism group, $^{a–c}$different letters in the same row indicate significant differences between samples (ripening stages) ($p < 0.05$)

KHDP date paste at kahlal stage, *RTDP* date paste at rutab stage, *TMDP* date paste at tamar stage, *nd* not detected

Date Waters

Date waters were obtained after soaking the date pastes in distilled water and the separation of solids by filtering. Each date paste was subjected to 3 consecutive soakings, and the date water obtained after each soaking was separately analyzed. Figure 3 shows the TSS of all the date waters obtained after each consecutive soaking of date pastes at different ripening stages. As can be expected, TSS decreased ($p < 0.05$) with the number of soaks: the highest TSS content was obtained in date waters after the first soak and the lowest after the third one, regardless of the ripening stage. The high content of soluble sugars in date pastes easily passes into the water during the soaking stage; thus, the higher the sugar content of the original paste, the greater the flow (Zhou et al., 2015). This could explain the higher TSS content found in the date waters from the first soaking in comparison with the second and third ones. Regarding the ripening stage, it could be said that the riper, the higher the TSS content in date water, although not in all cases these differences were statistically significant. RTDW1 and TMDW1 showed the highest TSS values (18.3–19.5°Brix) which are very close to the TSS content reported for date juice freshly prepared (20°Brix) (Kulkarni et al., 2010).

Mineral content of date waters is presented in Table 4. As expected, there is a coincidence between the majority of minerals in the date pastes (Table 1) and those in the date waters (K, Mg, and Ca). The higher the concentration in the original date paste, the higher the extraction with water. These three minerals have been reported as the main minerals in several fruit juices (lemon, orange, grape, mango, pineapple, watermelon, etc.) (Ichado & Ayeni, 2020; Oladipo et al., 2022). It should be noted that K was the only mineral that exceeds the amount of 1 g/L in some date waters, specifically in date water from the first soaking (KHDW1, RTDW1, and TMDW1). The content of all minerals decreased significantly ($p < 0.05$) with the number

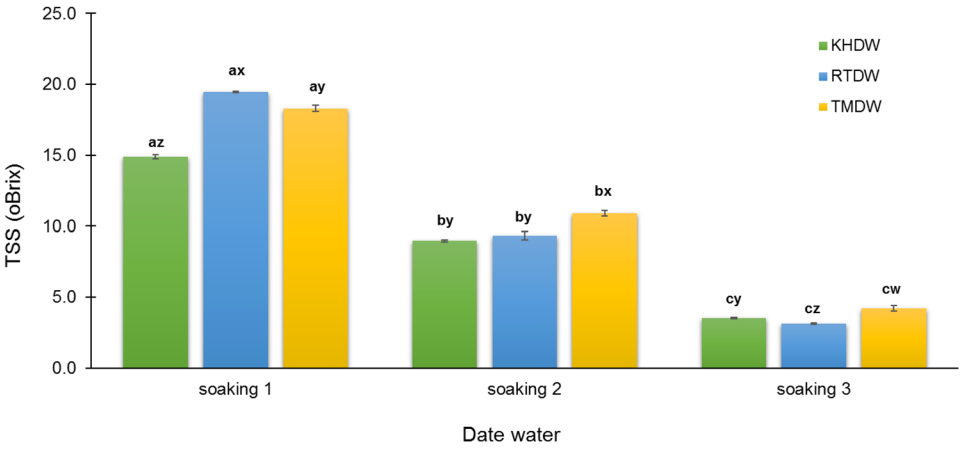


Fig. 3 Total soluble solids (°Brix) of date waters obtained after each of the three consecutives soaking of date pastes at different ripening stages (kahlal, rutab, and tamar). For the same sample, (a–c) different letters indicate significant differences between soaking ($p < 0.05$); for the same soaking, (x–z) different letters indicate significant differences between samples

of soakings, except for Fe in KHDW ($p > 0.05$), supporting the fact that the major extraction of minerals occurs during the first soaking. Based on this fact, it could be said that date waters might be considered a good source of minerals.

Sugars along with organic acids are the main soluble components of ripe fruits being easily extracted with water. During the soaking of date pastes, great amount of sugars was extracted with the water (Table 5). The amount of extracted sugars was the highest in the first soak progressively decreasing in the following soaks ($p < 0.05$), which agree with TSS results (Fig. 3). The highest sugar contents correspond to waters from the ripest pastes ($p < 0.05$). The average values of sugars (glucose + fructose) extracted after the 3 soakings of the pastes in increasing order of ripening were: 35.0 g/100 mL in KHDW, 45.9 g/100 mL in RTDW, and 51.7 g/100 mL in TMDP. No clear pattern was observed regarding the higher or lower extraction of glucose vs. fructose in relation to the ripening stage or the number of soakings. Glucose and fructose were detected as the main sugars in citrus water (from coproducts of juice industries) in amounts of 200 g/L and 125 g/L, respectively (Viuda-Martos et al., 2010), and in quince water in amounts of 6.0 g/kg and 14.2 g/kg, respectively (Trigueros et al., 2011). Overall, the results showed that date waters are and excellent source of natural sugars with potential application as carbon source for microbial flora (i.e., in fermented foods) or as a sweetener for the food industry.

With regard to the organic acids the main ones extracted were oxalic, malic, succinic, and citric acids (Table 5). Tartaric acid was not detected in TMDW (at any soaking) or in KHDW in the second and third soaking. For each date water, the amount of acids decreased progressively with soaking ($p < 0.05$). The maximum total acidity corresponded to TMDW1 (0.55 g/100 mL) and the minimum to RTDW3 (0.14 g/100 mL). Trigueros et al. (2011) and Viuda-Martos et al. (2010) reported that waters from citrus and quince

Table 4 Mineral profile (mg/L) of date waters obtained after three consecutive soaking of date pastes at different ripening stages (kahlal, rutab, and tamar)

Date water	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
KHDW1	152.98 ± 2.12^{a}	0.48 ± 0.02^{a}	0.75 ± 0.07^{a}	1010.02 ± 14.14^{a}	212.50 ± 0.71^{a}	0.76 ± 0.02^{a}	19.70 ± 0.28^{a}	0.70 ± 0.14^{a}
KHDW2	90.75 ± 0.21^{b}	0.33 ± 0.03^{b}	0.85 ± 0.08^{a}	614.50 ± 21.92^{b}	110.50 ± 3.64^{b}	0.43 ± 0.01^{b}	14.55 ± 0.21^{b}	0.67 ± 0.09^{a}
KHDW3	54.50 ± 2.55^{c}	0.17 ± 0.01^{c}	0.82 ± 0.12^{a}	238.00 ± 12.73^{c}	33.15 ± 0.78^{c}	0.18 ± 0.01^{c}	3.53 ± 0.25^{c}	0.25 ± 0.06^{b}
RTDW1	178.54 ± 3.54^{a}	1.25 ± 0.01^{a}	1.44 ± 0.14^{a}	1425.04 ± 7.07^{a}	269.00 ± 4.24^{a}	0.55 ± 0.01^{a}	8.76 ± 1.04^{a}	0.84 ± 0.04^{a}
RTDW2	110.50 ± 6.36^{b}	0.68 ± 0.06^{b}	0.86 ± 0.18^{b}	813.50 ± 3.54^{b}	137.50 ± 6.36^{b}	0.32 ± 0.06^{b}	4.66 ± 0.16^{b}	0.61 ± 0.09^{b}
RTDW3	48.35 ± 6.86^{c}	0.27 ± 0.01^{c}	0.60 ± 0.02^{c}	297.00 ± 2.83^{c}	41.30 ± 0.28^{c}	0.14 ± 0.01^{c}	1.82 ± 0.01^{c}	0.28 ± 0.10^{c}
TMDW1	155.00 ± 3.78^{a}	1.02 ± 0.01^{a}	1.12 ± 0.03^{a}	1300.06 ± 113.14^{a}	252.50 ± 7.78^{a}	0.58 ± 0.01^{a}	5.49 ± 0.65^{a}	0.74 ± 0.09^{a}
TMDW2	94.65 ± 3.04^{b}	0.64 ± 0.01^{b}	0.91 ± 0.01^{b}	732.50 ± 42.98^{b}	130.50 ± 4.85^{b}	0.38 ± 0.01^{b}	4.17 ± 0.47^{b}	0.59 ± 0.05^{b}
TMDW3	48.15 ± 4.60^{c}	0.34 ± 0.01^{c}	1.06 ± 0.07^{a}	321.00 ± 14.14^{c}	41.95 ± 1.20^{c}	0.21 ± 0.01^{c}	1.96 ± 0.07^{c}	0.40 ± 0.11^{c}

For the same sample (KHDW, RTDW, or TMDW) and mineral, $^{a–c}$different letters in columns indicate significant differences between soaking ($p < 0.05$)

KHDW1 date water at kahlal stage from the first soaking, *KHDW2* date water at kahlal stage from the second soaking, *KHDW3* date water at kahlal stage from the third soaking, *RTDW1* date water at rutab stage from the first soaking, *RTDW2* date water at rutab stage from the second soaking, *RTDW3* date water at rutab stage from the third soaking, *TMDW1* date water at tamar stage from the first soaking, *TMDW2* date water at tamar stage from the second soaking, *TMDW3* date water at tamar stage from the third soaking

Table 5 Sugar and organic acid profile (mg/100 mL) of date waters obtained after three consecutive soaking of date pastes at different ripening stages (kahlal, rutab, and tamar)

	Sugars (mg/100 mL)			Organic acids (mg/100 mL)			
Date water	Glucose	Fructose	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Succinic acid
KHDW1	6918.60 ± 14.21^{aY}	7468 ± 100.34^{aX}	253.22 ± 0.05^{aA}	4.59 ± 0.05^{aD}	1.06 ± 0.05^{E}	95.82 ± 0.73^{aB}	39.82 ± 0.44^{aC}
KHDW2	5281.09 ± 299.65^{bX}	5914.34 ± 117.39^{bX}	193.83 ± 1.44^{bA}	2.70 ± 0.89^{bD}	nd	68.92 ± 0.53^{bB}	24.52 ± 0.56^{bC}
KHDW2	5684.52 ± 279.54^{bX}	3741.85 ± 1.31^{cY}	114.89 ± 2.02^{cA}	2.95 ± 0.00^{bD}	nd	24.36 ± 0.057^{cB}	15.47 ± 0.06^{cC}
RTDW1	9539.85 ± 45.90^{aY}	10,346.38 ± 37.41^{aX}	285.52 ± 2.43^{aA}	4.25 ± 0.26^{aD}	3.19 ± 0.73^{aD}	151.91 ± 0.98^{aC}	41.50 ± 0.96^{aB}
RTDW2	9574.31 ± 175.81^{aX}	6763.45 ± 126.36^{bY}	178.01 ± 3.93^{bA}	3.12 ± 0.31^{bD}	2.54 ± 0.09^{bD}	90.93 ± 0.34^{bB}	27.41 ± 0.62^{bC}
RTDW3	6249.51 ± 64.93^{bX}	3397.67 ± 14.56^{cY}	81.90 ± 0.08^{cA}	1.45 ± 0.04^{cD}	1.83 ± 0.12^{cD}	47.53 ± 10.67^{cB}	11.78 ± 0.75^{cC}
TMDW1	9367.91 ± 426.12^{aY}	10,430 ± 182.21^{aX}	334.86 ± 3.17^{aA}	5.58 ± 0.17^{aD}	nd	160.94 ± 2.87^{aB}	48.46 ± 1.71^{aC}
TMDW2	9508.09 ± 234.10^{aX}	8844.52 ± 113.30^{bY}	219.03 ± 0.68^{bA}	4.69 ± 0.05^{bD}	nd	105.73 ± 0.86^{bB}	27.41 ± 0.29^{bC}
TMDW3	6400.42 ± 170.17^{bY}	7173.54 ± 94.24^{cX}	86.88 ± 0.76^{cA}	1.83 ± 0.07^{cD}	nd	49.86 ± 0.45^{cB}	11.91 ± 0.07^{cC}

For the same sample (KHDW, RTDW, or TMDW) and compound, $^{a–c}$different letters in columns indicate significant differences between soaking ($p < 0.05$). For the same sample, $^{X, Y}$different letters in the same row indicate significant differences between sugars ($p < 0.05$) and $^{A–E}$different letters in the same row indicate significant differences between organic acids ($p < 0.05$)

KHDW1 date water at kahlal stage from the first soaking, *KHDW2* date water at kahlal stage from the second soaking, *KHDW3* date water at kahlal stage from the third soaking, *RTDW1* date water at rutab stage from the first soaking, *RTDW2* date water at rutab stage from the second soaking, *RTDW3* date water at rutab stage from the third soaking, *TMDW1* date water at tamar stage from the first soaking, *TMDW2* date water at tamar stage from the second soaking, *TMDW3* date water at tamar stage from the third soaking, *nd* not detected

were able to extract a great part of organic acids from fruit pastes. These authors reported a total acidity of 0.43 g/100 g in quince water and 0.52 g/100 mL in citrus water.

Date Flour

Proximate composition of the three date flours is shown in Table 6. The main components in all flours were TDF (58–66%) and sugars (19–26%) following the same trend observed in pastes affected by the ripening stage: KHDF and RTDF showed the lowest TDF content but the highest sugar content, in opposition to TMDF (the ripest) that showed the lowest sugar content but the highest TDF content ($p < 0.05$). The higher extraction of sugars in the date waters of the ripest pastes (TMDP) could be contributing to this pattern. Moisture (4.6–7.3%), protein (6.8–7.4%), and ash content (1.3–1.9%) were not affected by the ripening stage. Flours showed a low fat content (< 1.2%) with slight differences between samples. Several authors have reported extraction processes to obtain dietary fiber concentrates with higher TDF content (> 90%) and lower sugar content (< 1%) than our flours but applying hot water and a greater number of washes or soaks (i.e., 7 times) (Borchani et al., 2010; Hasnaoui et al., 2012) than those used in the present study, resulting in higher water and energy consumption. In this case, the purpose was not to obtain a sugar-free flour or powder but an intermediate and stable ingredient with potential suitability to be used in the development of new foods.

Regarding mineral content (Table 6) it could be said that in general, date flours followed the same pattern as the one observed in date pastes (K and Mg as the main minerals and Zn and Mn the minorities) (Table 1) but with different concentrations, depending on their initial content and on the greater or lesser extraction with date waters. For instance, although potassium continues to be the major mineral, being the most extracted in the date water (Table 4), its concentration in the flour is lower than in the pastes. Like what has been previously stated for date pastes, flour can also be considered a good source of macroelements but poor in microelements. Higher amounts of Fe, Cu, Zn, and Mg have been reported in wheat flour, rice flour, hemp flour, corn flour, barley flour, rye flour, and oat flour (Leśniewicz et al., 2009; Rusu et al., 2021; Tian et al., 2022).

Sugar and organic acid profile of date flours is shown in Table 6. All date flours showed higher fructose than glucose content ($p < 0.05$), in contrast to pastes (Table 1), which could be explained by the higher extraction of glucose than fructose in date waters (Table 5). All flours presented lower organic acid content than the corresponding pastes (which was expected due to their extraction in date waters) but with different proportions of each organic acid. Oxalic and malic acids were detected in the three flours, with malic acid being the major one ($p < 0.05$). Tartaric acid was not detected in KHDF, and succinic and citric acids were not detected in TMDF. TMDF showed the lowest total acidity (0.26 g/100 g), followed by RTDF (0.72 g/100 g) and KHDF (0.74 g/100 g) representing reductions between 65 and 82% of the total acidity of the corresponding pastes.

Physicochemical properties (pH and color properties) of date flours affected by the ripening stage of the original dates are shown in Table 7. All flours were mildly acidic with pH ranging from 6.22 (TMDF) to 6.36 (KHDF). Slightly lower

Table 6 Proximate composition and mineral, sugar, and organic acid profile of date flours from dates at different ripening stages (kahlal, rutab, and tamar)

	KHDF	RTDF	TMDF
Proximate composition (g/100 g)			
Moisture	4.62 ± 0.33[a]	7.26 ± 1.30[a]	5.69 ± 1.01[a]
Fat	0.53 ± 0.09[b]	1.07 ± 0.55[a]	1.15 ± 0.73[a]
Protein	7.38 ± 0.3[a]	6.75 ± 0.10[a]	6.78 ± 0.65[a]
Ash	1.93 ± 0.33[a]	1.34 ± 0.50[a]	1.66 ± 0.26[a]
TDF	60.14 ± 1.98[b]	57.64 ± 1.27[b]	65.98 ± 1.38[a]
Sugars	25.40 ± 0.26[a]	25.94 ± 0.54[a]	18.74 ± 0.82[b]
Mineral composition (mg/100 g)			
Ca	280.34 ± 5.58[a]	233.39 ± 3.54[b]	272.79 ± 6.85[a]
Cu	2.14 ± 0.04[a]	1.18 ± 0.00[c]	1.71 ± 0.02[b]
Fe	3.52 ± 0.08[b]	3.34 ± 0.05[c]	4.24 ± 0.01[a]
K	395.31 ± 8.88[a]	398.29 ± 9.02[a]	359.36 ± 12.96[b]
Mg	185.85 ± 1.85[a]	164.92 ± 0.24[b]	165.05 ± 6.05[b]
Mn	1.59 ± 0.05[c]	2.22 ± 0.04[b]	2.45 ± 0.01[a]
Na	8.42 ± 0.43[a]	5.31 ± 0.19[c]	6.77 ± 0.56[b]
Zn	1.37 ± 0.02[a]	1.30 ± 0.03[b]	1.40 ± 0.02[a]
Sugars (mg/g)			
Glucose	111.13 ± 3.68[a]	112.68 ± 4.27[a]	76.37 ± 1.08[b]
Fructose	129.56 ± 2.28[b]	141.35 ± 0.52[a]	94.15 ± 1.35[c]
Organic acids (mg/g)			
Oxalic acid	0.12 ± 0.00[c]	0.53 ± 0.00[a]	0.36 ± 0.01[b]
Citric acid	0.40 ± 0.05[b]	1.15 ± 0.10[a]	nd
Tartaric acid	nd	0.52 ± 0.03[a]	0.28 ± 0.02[b]
Malic acid	5.26 ± 0.16[a]	2.83 ± 0.13[b]	1.95 ± 0.04[c]
Succinic acid	1.64 ± 0.16[b]	2.19 ± 0.04[a]	nd

For the same compound, [a–c]different letters in the same row indicate significant differences between samples (ripening stages) ($p < 0.05$)

KHDF date flour at kahlal stage, *RTDF* date flour at rutab stage, *TMDF* date flour at tamar stage, *nd* not detected

pH through ripening progress could be related to a higher extraction of organic acids (toward the date water through the soaking process) from the corresponding date paste. The pH values of date flours were higher than the corresponding pH of date pastes (Table 2), which may indicate that the soluble organic acids of the date pastes were mostly extracted in the date water. Majzoobi et al. (2019) reported that the soluble organic acids of date fruits were mostly extracted in the juice resulting in higher pH values of the date press cake than the ones of date juice. Regarding color parameters, it could be said that the three date flours differ only in terms of lightness and hue, because although significant differences ($p < 0.05$) were detected for all the color parameters evaluated, the variations between samples for the a^* and b^* coordinates and the saturation (C^*) were less than 1 unit and therefore without practical meaning. Date flour lost lightness as the ripening stage advances, with KHDF being the lightest and TMDF the darkest. In this case, the development of darker color could possibly be attributed to non-enzymatic browning (Maillard and caramelization reactions) (Hasan et al., 2022) induced by heat treatment during flours processing (Fig. 1) which would be more probable at higher sugar content (corresponding with the ripest stages). This effect could also be responsible for the decrease of hue values toward yellow-orange hues.

Table 7 Physicochemical properties of date flours from dates at different ripening stages (kahlal, rutab, and tamar)

Parameter	KHDF	RTDF	TMDF
pH	6.36 ± 0.01[a]	6.32 ± 0.01[b]	6.22 ± 0.03[c]
L^*	62.46 ± 0.27[a]	61.97 ± 0.25[a]	56.72 ± 0.13[b]
a^*	3.93 ± 0.04[a]	3.15 ± 0.07[b]	3.33 ± 0.09[b]
b^*	13.44 ± 0.08[b]	12.96 ± 0.07[c]	13.89 ± 0.12[a]
C^*	14.01 ± 0.08[b]	13.34 ± 0.07[c]	14.28 ± 0.14[a]
H^*	73.71 ± 0.19[b]	76.33 ± 0.28[a]	76.54 ± 0.24[a]

For the same parameter, [a–c]different letters in the same row indicate significant differences between samples (ripening stages) ($p < 0.05$)

KHDF date flour at kahlal stage, *RTDF* date flour at rutab stage, *TMDF* date flour at tamar stage

Hydration properties of date flours (WHC and SWC) (Fig. 4) decreased through the ripening process reaching the highest values for KHDFDF and the lowest ($p < 0.05$) for RTDF and TMDF (without differences between them). These flours due to their powder structure have the ability to hold water depending on the moisture gradient between the powder and the surrounding. Date flour with the lowest moisture content (KHDF, Table 6) and therefore with the highest moisture gradient when it is in contact with water, it was expected to show the highest water absorption and the highest WHC and SWC values. Similar behavior has already been reported for dehydrated fruits and food powders (Ferrari et al., 2012; Hasan et al., 2022). For the same reason, WHC and SWC of date flours are higher than those of the corresponding date pastes (Fig. 2). All the date flours showed similar OHC ($p > 0.05$) with a mean value of 1.37 g oil/g flour (Fig. 4) which allow them to be used as carrier of oils (i.e., with healthy lipid profile) or as an emulsion stabilizer in the development of functional foods.

The microbiological quality of date flours was significantly improved in comparison with the corresponding date flours. Total aerobic bacteria were not detected in any flour. Yeasts were only detected in RTDF (0.30 log CFU/g), and molds in RTDF (0.39 log CFU/g) and KHDF (0.45 log CFU/g), without differences between them ($p > 0.05$). Counts lower than 0.7 log CFU/g were obtained for *Enterobacteriaceae*, without differences between samples ($p > 0.05$). These differences could be due to the different sensitivities to heating and drying processes of the different analyzed microorganisms (Alp & Bulantekin, 2021).

Given the special characteristics of the different intermediate food products with high added value obtained from the

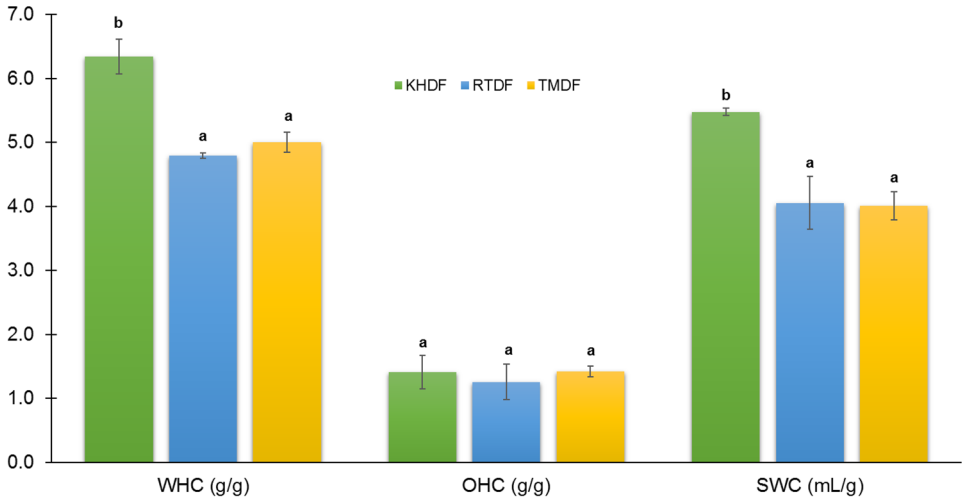


Fig. 4 Techno-functional properties (WHC, water holding capacity; OHC, oil holding capacity; SWC, swelling capacity) of date flours from dates at different ripening stages (kahlal, rutab, and tamar). For the same parameter, (a, b) different letters indicate significant differences between samples ($p < 0.05$)

valorization of date co-products (pastes, waters, and flours), in order to ensure their food safety, preservation methods in line with these characteristics should be applied. For example, date pastes and waters, due to their high moisture and sugar content, should be kept frozen/refrigerated, while date flours, due to their high hygroscopicity, could be kept vacuum packed and/or refrigerated. In any case, studies should be carried out to determine their shelf life under specific storage conditions. In addition, for their application as ingredients in foods, processing conditions should be optimized to ensure food safety.

In view of the results obtained, further research should be directed toward the optimization of the processes for their addition in different food matrices. For example, date pastes could be integrated into meat and dairy products as well as into bakery and pastry products; the first date waters could be useful for the development of syrups, liquid caramel, etc.; date waters 2 for the development of fresh fruit drinks or as an ingredient in the development of fermented meat products; and date waters 3 would have potential application in bakery, confectionery, reconstitution for the dairy industry, among others; lastly, date flours could be used bakery, pastry, and meat products.

Conclusions

The valorization of the co-products generated from the commercialization of fresh dates (Confitera cv.) applying simple and environmentally friendly technologies is a technologically viable strategy. Their application has resulted in three types of high added-value products (date pastes, date waters, and date flours) with different appearance, physicochemical properties, and composition, giving them great versatility for their incorporation in different food matrices, depending on both the technological objective to be achieved and the characteristics aimed in the new product developed. This research contributes to the reduction of agro-industrial waste, to the sustainability of fresh date production in Spain, to economic and social development in this specific area, and therefore to the circular economy.

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Data Availability Data are available on request from the corresponding author.

Declarations

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7.3. PUBLICACIÓN 3

In vitro evaluation of biological properties of high-added value ingredients (date juice and date powder) obtained from date co-products

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In vitro evaluation of biological properties of high-added value ingredients (date juice and date powder) obtained from date co-products

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ABSTRACT

The commercialization of fresh dates results in a significant amount of waste, with approximately 30 % of dates being discarded due to low-grade classification. To combat food waste, value-added products, such as previously dried and milled date powder and date juice, have been obtained from date co-products using environmentally friendly processes. Fresh dates contain a number of valuable nutritional components, including sugars, dietary fibre, essential vitamins, minerals, and bioactive compounds. This study aimed to assess the chemical composition of date juice and powder, in terms of total soluble solids, total dietary fibre, sugars, proteins, moisture, ash and fats. In addition, the total phenolic compounds content was determined, and *in chemico* antioxidant, antidiabetic and antihypertensive activities, and prebiotic potential were evaluated. In terms of nutritional values, date juice was found to be rich in water-soluble sugars, while date powder presented high concentrations of total dietary fibre. The nutritional composition strongly influenced the total phenolic compounds content (1.575 ± 0.028 mg GAE/g in date powder vs 0.146 ± 0.004 mg GAE/mL in date juice) and bioactivities (antioxidants, antidiabetic and antihypertensive activities), with date powder showing higher values compared to date juice. Prebiotic potential was observed for both by-products for all the strains tested. In this sense, both date juice and date powder proved to be valuable by-products developed to combat food waste.

1. Introduction

Currently, one of the primary challenges in the food industry is to promote the development of healthy and sustainable foods. The food industry aims to generate fewer waste products and to valorise the co-products generated during food production and processing. Furthermore, these new foods should help reduce the risk of several illnesses, particularly non-communicable chronic diseases associated with modern lifestyles, such as diabetes and hypertension, important public health problems worldwide responsible for widespread morbidity and mortality. For example, 1.5 million deaths are directly attributed to diabetes each year (World Health Organization (WHO), 2024)). Over the past few decades, both the incidence and prevalence of these diseases have consistently risen.

In this context, being able to identify foods or ingredients with specific biological value (namely rich in antioxidants, antidiabetic compounds, antihypertensive agents or prebiotics, among others) to combat such prevalent health conditions, such as diabetes and hypertension, is crucial. Oxidative stress plays a crucial role in the development and progression of both these illnesses, and natural antioxidants present in certain foods may help neutralize harmful free radicals, reducing oxidative damage to cells and tissues (Chaudhary et al., 2023). In addition, certain bioactive compounds found in foods have been found to exhibit antidiabetic effects contributing to better glycemic control and reduced diabetes risk or antihypertensive properties which may help manage blood pressure and overall cardiovascular health. Not less important, is gut health, increasingly recognized as a key factor in preventing and managing chronic diseases. Prebiotics and non-digestible food compounds contribute to this context by selectively enhancing the growth of probiotics and supporting human health

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through nutrient enrichment, as well as by modulating beneficial gut microbiota and the immune system. Consuming foods rich in prebiotic compounds supports gut health and may indirectly impact diabetes and hypertension management (Megur et al., 2022). Taking into account the complexity and variety of the chemical components present in different food types, and the diversity of interactions in the biochemical networks and biological systems, metabolomic approaches will be essential in advancing nutritional food research (Emwas et al., 2021).

For millennia, dates (*Phoenix dactylifera*) have been a staple in the diets of inhabitants of different regions (mainly Arabian Peninsula and the Sahara Desert in North Africa) where local cultures have developed extensive knowledge of the benefits of dates, recognizing them as a rich source of essential nutrients which is highly influenced by variety or cultivar and growth conditions and areas (Alsuhaymi et al., 2023). The production and marketing of fresh dates in Spain has significantly increased due to promotional campaigns highlighting their growth characteristics. The Confitera cultivar, autochthonous and well-suited to the unique soil and climate conditions of the European oasis, demonstrates eco-efficient growth and supports sustainable production. Additionally, promotional campaigns have highlighted the nutritional and health benefits of dates (Fernández-López et al., 2022). Fresh dates contain valuable nutritional components such as sugars, dietary fibre, essential vitamins, and minerals, as well as bioactive compounds including polyphenolic compounds, anthocyanins, sterols, and carotenoids (Hussain et al., 2020; Muñoz-Bas et al., 2023). Furthermore, dates are a product deeply connected to the region, playing a vital role in the development of the local economy and serving as a prime example of a circular economy.

However, their commercialization leads to a substantial amount of coproducts, with around 30 % of dates being discarded due to low-quality classification based on factors such as size, colour, insects, or natural damages among others (Fernández-López et al., 2022) which usually end up as waste to be disposed of, with the consequent environmental risk. Various value-added products, such as rich fibre concentrates, date powder, date paste, date extracts and date juice, have been obtained from date co-products using environmentally friendly processes. These products have specific characteristics that make them suitable as functional ingredients in the food industry (Muñoz-Tebar et al., 2023). This approach has the potential to greatly minimize food waste in alignment with the UN's sustainable development objectives and support the circular economy.

Date juice and date powder are two of these value-added ingredients obtained from date co-products applying simple and environmentally friendly technologies (Muñoz-Bas et al., 2024). Both have different appearances, physicochemical properties, and compositions, including moisture, sugars, dietary fiber, organic acids, minerals and bioactive compounds. This provides them with significant flexibility for integration into various food matrices, based on the technological goals to be met and the specific attributes of the new product being created. For instance, date juices could be utilized in crafting fresh fruit beverages, syrups and liquid caramel. It could also be used as an ingredient in the development of fermented meat products, as well as in bakery, confectionery and reconstitution medium for the dairy industry. Similarly, date powder could be used as an ingredient for the development of bakery, pastry, and meat products (Muñoz-Bas et al., 2024). Considering the multitude of food applications of date juice and powder, the evaluation of their biological activities, such as antioxidant antidiabetic, antihypertensive and prebiotic activities, will be of great importance for demonstrating their potential beneficial effects on human health.

Based on the above rationale, the aim of this work was to provide, for the first time, an in-depth characterization of the chemical composition (total soluble solids (TSS), total dietary fibre (TDF), total sugars, proteins and fat, moisture, ash and total phenolic content) and of associated biological activities (antioxidant, antidiabetic, antihypertensive and prebiotic properties) of date juice and date powder, which are added-value ingredients derived from date coproducts.

2. Materials and methods

2.1. Preparation of high added-value ingredients from date co-products

Date co-products (non-commercial or discard dates due to externally damaged, undersized, etc.) from the industrialization of fresh dates (Confitera cv.) harvested at the tamar stage from the Elche Palm Grove (Elche, Alicante, Spain) were provided by the Catedra Palmeral d'Elx (UMH, Alicante, Spain). These co-products were transported under refrigerated conditions (6 ± 2 °C) to the Food Pilot Plant of the Orihuela Polytechnic School (EPSO) of the Miguel Hernández University and immediately processed following the procedure described by Muñoz-Bas et al. (2024) to obtain date juice and date powder, applying only physical treatments. Briefly, the dates were pitted and grounded and the resulted paste was soaked several times with distilled water- Then the juice date was obtained by pressing through cotton filter clothes and the powder date by drying the remaining solid part in an oven (60 ºC, 24 h). Date juice was packaged in opaque glass containers and frozen at -18 °C, while the date powder was vacuum-packed in plastic bags protected from light. Part of the conditioned products (date powder and date juice) were analysed for chemical composition whereas a second part was transported to the laboratories of the Catholic University of Porto (Porto, Portugal) to carry out the remaining chemical analyses (total phenolic content) and the assessment of the antioxidant, antidiabetic, antihypertensive and prebiotic biological activities.

2.2. Chemical analysis of high added-value ingredients

Date powder was analyzed in triplicate for moisture (AOAC 925.45), total protein (AOAC 981.10), total fat (AOAC 991.36), ash (AOAC 923.03), and total dietary fiber (AOAC 985.29) contents following AOAC methods (AOAC 2006). Moisture content was measured by drying 2 g of sample in a vacuum oven. Protein content was estimated from the analysis of the nitrogen content through the Kjeldahl method (Bloc digest 12 (Selecta, Barcelona, Spain), and Kjeltec 8400 analyzer unit (Foss Hillerod, Denmark), using a conversion factor of 6.25. The ash content was assessed by the incineration of 2 g of sample at 550 ºC until the total elimination of organic matter. The fat content was determined according to the Soxhlet extraction principle using a semiautomatic extractor (SOXTHERM SOX, Gerhardt GmbH & Co., Königswinter, Denmark). Total dietary fiber was assessed by the enzymatic-gravimetric method using the GDE enzymatic digester and CSF filtration system (Velp Scientifica, Usmate, Italy). Total sugar content was calculated by difference, subtracting the sum of the other components (moisture, protein, fat, ash, and dietary fiber) from the total (100 %).

Total soluble solids (TSS) content of date juice was determined using a digital refractometer Milwaukee MA 871 (Milwaukee electronics, Milwaukee, WI, USA) and expressed as ^{0}Brix.

2.3. Date powder extracts preparation

The date powder extracts were prepared according to the method described by Hung et al. (2011), with minor modifications. Initially, 20 mL of 80 % ethanol, previously prepared by diluting absolute ethanol (VWR, PA, USA), were added to 2 g of the date powder sample. The solution was homogenised by agitation in an orbital shaker (Orbital Shaker Wiggenhouse, Berlin, Germany) for 20 min at 200 rpm at a temperature of 30 °C, followed by sonication (Bath sonicator, Bandelin, Berlin, Germany) for 10 min. Subsequently, the sample was subjected to centrifugation at 3850×g for 5 min at 20 °C (Hettich Universal 320R Centrifuge, Andreas Hettich GmbH & Co.). The supernatant was collected in a new 50 mL tube and the procedure was repeated by adding 10 mL of 80 % ethanol. The samples were concentrated using a rotavapor (Buchi, Flawil, Switzerland). Each sample was subjected to a 30 min exposure to the rotavapor at a temperature of 45 °C and a pressure of 100 atm. A final volume of 5 mL of sample was obtained.

2.4. Total phenolic content determination

The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method, following the procedure described by Coscueta et al. (2018), with minor amendments. A standard curve of gallic acid (0.025–0.200 mg/mL) was created to present the findings in mg gallic acid equivalents per mL of sample (mg GAE/mL). Using a 96-well microplate, 30 μL of each sample (or suitable dilution), 100 μL of Folin-Ciocalteu reagent (20 % v/v) (Merck KGaA, Darmstadt, Germany), and 100 μL of anhydrous sodium carbonate solution (7.4 % w/v) were pipetted into each well. The microplate was then incubated, wrapped in aluminium foil, for 30 min at 25 °C in darkness. The resulting mixture was measured at 765 nm using a multi-detection plate reader (Synergy H1, VT, USA) with Gen5 software. All measurements were conducted in triplicate.

2.5. The 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging assay

The antioxidant activity was evaluated using the 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay (ABTS) as outlined by Gonçalves et al. (2009), with minor adjustments. Initially, the ABTS working solution's concentration was modified to achieve an absorbance of 0.70 (± 0.02) at 734 nm. To create the Trolox solution, 0.0125 g of Trolox (Sigma-Aldrich, MO, USA) was measured and dissolved in 1 mL of methanol (Fischer Chemical, MA, USA), then diluted to 50 mL final volume with deionised water. The results were expressed in Trolox equivalents, utilising a standard curve (25 μM-175 μM). For the assay, 20 μL of Trolox or sample and 180 μL of ABTS solution were added to each well of a 96-well microplate. The microplate was subsequently incubated for 5 min at 30 °C and the absorbance was measured at 734 nm using a Multi-detection plate reader (Synergy H1, VT, USA). All analyses were conducted in triplicate.

2.6. Prebiotic activity

Date juice and date powder were assessed for their potential to promote growth and metabolic activity of different probiotic strains. For date juice, being a liquid matrix, the prebiotic potential was assessed using the 96-well microplate method (microscale) determining cell growth via absorbance, while for date powder, being a solid matrix, the prebiotic potential was assessed at macroscale determining cell growth by viable cell numbers enumeration using colony-forming unit (CFU) counts.

2.6.1. Growth media preparation

The basal medium used for the evaluation of the bifidogenic potential of date juice and date powder was Man-Rogosa-Sharpe broth (MRS) prepared by mixture of the different components to allow carbon source substitution. The detailed composition of MRS broth is described in Table 1.

From this basal medium the following media were prepared:

1. MRS basal broth without glucose, as negative control
2. MRS with 2 % (w/v) glucose (Fluka, Charlotte, NC, USA), as positive control
3. MRS with 2 % (w/v) fructooligosaccarides (FOS) (Orafti, Oreye, Belgium), as positive prebiotic control.
4. MRS with 2 % and 10 % (v/v) of date juice
5. MRS with 2 % and 6 % (v/v) of date powder

The two concentrations of date juice and date powder tested were established based on the respective total sugars content, to approach that found in MRS medium. In the case of *Bifidobacterium* stains all media were subsequently supplemented with filter-sterilized 0.5 g/L of l-cysteine·HCl (Alfa Aesar, Kandel, Germany).

Table 1
Detailed composition of Man-Rogosa-Sharpe basal medium.

Compound	Source	Concentration (g/L)
Tryptone	Sigma-Aldrich (St. Louis, MI, USA)	10
Meat extract	Merck (Darmstadt, Germany)	8
Yeast extract	Biokar Diagnostics (Allone, France)	4
Di-potassium hydrogen phosphate	Merck (Darmstadt, Germany)	2
Tween 80	Merck (Darmstadt, Germany)	1
Sodium acetate	Merck (Darmstadt, Germany)	5
Ammonium citrate tribasic	Sigma-Aldrich (St. Louis, MI, USA)	2
Magnesium sulfate	Merck (Darmstadt, Germany)	0.2
Manganese sulfate	Sigma-Aldrich (St. Louis, MI, USA)	0.04
Carbon source	-	20

2.6.2. Bacterial growth

For the microplate assay, 11 probiotic strains were selected for screening including, *Lactobacillus acidophilus* KI (CSK, Ede, Netherlands), *Lacticaseibacillus paracasei* L26 (Christian (Chr) Hansen, Hørsholm, Denmark), *Lacticaseibacillus rhamnosus* R11 (Lallemand, Montréal, QC, Canada), *Lacticaseibacillus casei* 01 (Chr. Hansen, Hørsholm, Denmark), *Lactobacillus acidophilus* La-5 (Chr. Hansen, Hørsholm, Denmark), *Lactiplantibacillus plantarum* 299v (Probi AB, Lund, Sweden), *Bifidobacterium animals* subspecies *lactis* BB-12® (Chr. Hansen, Hørsholm, Denmark), *Bifidobacterium breve* NCIMB, *Bifidobacterium animalis* Bo (CSK, Ede, Netherlands), *Bifidobacterium longum* BG3 (Cell Biotech, Hellerup, Denmark) and *Bifidobacterium animalis* BLC (DSM Food Specialties, Moorebank, Australia).

The probiotic strains with the best performance in the microplate assay were selected for the sequential macroplate assay, namely: L. *casei* 01, L. *rhamnosus* R11, *B. breve* NCIMB, *and B. animalis* BLC.

For each experiment, the bacterial strains were reactivated in the appropriate broth for 24 h. The lactobacilli strains were grown under aerobic conditions, while the bifidobacteria strains were grown under anaerobic conditions (85 % N_2, 5 % H_2, and 10 % CO_2), achieved using a Whitley A35 HEPA anaerobic workstation incubator (Bingley, United Kingdom), after thawing a glycerol stock. For the growth of *Lactobacillus* strains MRS medium was used, while for the growth of *Bifidobacterium* stains MRS medium was subsequently supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl (Alfa Aesar, Kandel, Germany). For all strains, a single sub-culturing step was carried out under identical growth conditions, with a final incubation volume of 10 mL of MRS (supplemented with 0.05% cysteine for Bifidobacterium) and 1 % (v/v) cell inoculation. The initial colony-forming unit (CFU) count in each inoculum suspension was determined by preparing serial dilutions in PBS (Sigma-Aldrich, St. Louis, MI, USA). Subsequently, 10 μL of each dilution were plated in triplicate on suitable media. Agar plates were incubated at 37 °C for 48 h under anaerobic or aerobic conditions for bifidobacteria and lactobacilli strains, respectively. Following incubation, CFU enumeration was conducted, and the results were expressed as mean ± standard deviation CFU/mL for bacterial suspensions.

2.6.3. Screening of bacteria growth via microplate assay

The screening of the bacterial growth for the 11 probiotic strains via the microplate assay was performed according to Sousa et al. (2015), with slight modifications. Each previously prepared medium (refer to Section 2.6.1) was inoculated with the corresponding probiotic strain at a concentration of 2 % (v/v), in triplicate. Subsequently, 250 μL of the probiotic incorporated-growth medium was transferred to each corresponding well of a 96-well microplate. To prevent the presence of oxygen, 50 μL of autoclave-sterilized liquid paraffin (Merck, Germany) was added to the wells containing bifidobacteria strains. Cellular growth was

monitored throughout 24 h by measuring the optical density (OD) of the cultures at 655 nm using a 680 Microplate Reader from Bio-Rad (Hercules, CA, USA) in conjunction with Microplate Manager 5.2.1 Software. A negative control was established using MRS without glucose, while a growth control and a positive control were established by supplementing the MRS broth with glucose and FOS, respectively. The specific growth rates were determined by calculating the slope of the trend line and the absorbance values in the log phase of the growth curves. The maximum growth (maximum absorbance) was assessed to compare the results of the different growth conditions.

2.6.4. Evaluation of bifidogenic potential via determination of viable cell numbers

In the case of the date powder, its bifidogenic potential was assessed by evaluating the growth behavior of the four previously selected commercial probiotic strains (refer to Section 2.6.3). Their growth and acidification capacities in each of the five previously described growth media was measured via enumeration of viable cell numbers and measurement of pH evolution, following the procedure outlined by Sousa et al. (2015), with minor modifications. Bacterial metabolism was evaluated by measuring pH using a pH-meter (Crison Instruments, Barcelona, Spain). Each medium was inoculated with the corresponding probiotic strain at a concentration of 2 % (v/v). The inoculated media were then transferred to sterile 2mL Eppendorf tubes (in triplicate), and incubated at 37 ºC for 24 h under aerobic and anaerobic conditions for lactobacilli and bifidobacteria strains respectively (according to conditions mentioned in 2.6.2). To ensure anaerobic conditions for the *Bifidobacterium* strains, the media were supplemented with filter-sterilized 0.5 g/L of l-cysteine·HCl. Sampling was performed at 0, 3, 6, 10 and 24 h. Decimal dilutions were prepared in PBS at each sampling interval, and 10 µL of each dilution were plated thrice on suitable media. Following incubation, CFU counting was conducted, and results were reported in CFU/mL.

2.7. Antidiabetic activity (α-glucosidase inhibition assay)

The antidiabetic activity was assessed by measuring the α-glucosidase inhibitory activity, following a modified version of the method described by Kwon et al. (2008). Initially, 50 µL of sample was combined with 100 µL of α-glucosidase solution (1.0 U/mL) diluted in 0.1 M phosphate buffer (pH 6.9) per well. The microplate was then incubated at 25 °C for 10 min. Following incubation, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer was added to each well. The mixtures were subsequently incubated at 25 °C for 5 min, and absorbance was measured at 405 nm using a Multi-detection plate reader (Synergy H1, VT, USA). For assay efficacy control, 50 µL of buffer solution served as a negative control, whilst 50 µL of acarbose at 10 mg/mL concentration was used as a positive control. All measurements were conducted in triplicate. The α-Glucosidase inhibition percentage was calculated using the following formula:

$$\alpha - Glucosidase\ inhibition\ (\%) = \left(\frac{\Delta Abs_{control} - \Delta Abs_{sample}}{\Delta Abs_{control}}\right) x100$$

2.8. Antihypertensive activity (Angiotensin-I converting enzyme (ACE)-inhibitory activity assay)

The antihypertensive effect was evaluated via an ACE-inhibitory activity test as outlined by Sentandreu and Toldra (2006), with slight changes. Each well received 40 µL of ultrapure water or ACE working solution (42 mU/mL), and the final well volume was brought to 80 µL using ultrapure water. Then, 160 μL of substrate solution (0.45 mM) was added and the resulting mixture incubated at 37 °C for 30 min to allow the enzyme reaction to occur. Throughout the 30 min, the resulting fluorescence was measured with excitation and emission wavelength at 350 and 420 nm, respectively, using a Multi-detection plate reader (Synergy H1, VT, USA). All measurements were performed in triplicate. The ACE inhibitory activity was calculated using the following formula:

$$iACE(\%) = ((F_{CTL} - F_{BLK}) - (F_{SPL} - F_{SPLB})) * \frac{100}{F_{CTL} - F_{BLK}}$$

2.9. Statistical analysis

Data from this investigation were analyzed using SPSS software version 17.0 (SPSS; Chicago, IL, USA). These data were expressed as the mean ± standard derivation (SD) of replicates. All experiments were made in triplicate. Parametric tests were conducted on the data, which were found to follow a normal distribution according to the Shapiro-Wilk test (normality test). A t-Student for independent samples test was performed in order to conduct a statistical comparison between date juice and date powder. Statistical differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition

The nutritional value of fruit juices is due to their content in carbohydrates (mainly sugars), minerals, vitamins, organic acids, and bioactive compounds, which amounts depend on the original fruit, extraction process and preservation method applied because some of these compounds are highly susceptible to degradation. Dates are no exception, and the co-products date juice and powder, obtained during the processing of fresh dates will vary in composition depending on several factors including the separation process, the maturity of the dates, the presence of enzymes and their activity levels, extraction temperature and the drying process. For example, the drying process will influence the date powder composition, including sugar concentration, antioxidants, and dietary fiber content.

Indeed, and according to the reported results the high content of soluble sugars in date fruits (Muñoz-Bas et al., 2023) was easily extracted and passed into the water during the elaboration process of date juice. The date juice showed a high Total Soluble Solid (TSS) content (18.3–19.5 ^{o}Brix) which is in agreement with TSS in freshly prepared date juice (Kulkarni et al., 2010). The main component found in date powder was Total Dietary Fibre (TDF) (66.0 ± 1.4 g/100 g), followed by total sugars (18.7 ± 0.8 g/100 g), total protein (6.8 ± 0.7 g/100 g), moisture (5.7 ± 1.0 g/100 g), ash (1.7 ± 0.3 g/100 g) and total fat (1.2 ± 0.7 g/100 g). Date fruits are considered excellent sources of carbohydrates, comprising 60 % to 80 % of their composition, which includes both soluble sugars and dietary fiber (Muñoz-Bas et al., 2023; Stojanovska et al., 2023). The TDF content in date fruits ranges between 5.3 and 13.4 % depending on cultivar, growth conditions and ripening stage, being insoluble fiber the main fraction (77–90 %, mainly lignin) (Stojanovska et al., 2023). In-depth studies of date fruit fibers have indicated that dates are a source of soluble dietary fibers, including fructan, pectin, galactomannan, arabinoxylan, and β-glucan, with varying levels of these components found among different cultivars (George et al., 2020; Dhahri et al., 2023).

3.2. Prebiotic activity

The impact of prebiotics and probiotics on human nutrition and health is well-established (Yadav et al., 2022). Both have been commonly used as functional ingredients in the development of functional foods. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), prebiotics are “substrates that are selectively utilized by host microorganisms conferring a health benefit”. This group includes compounds of very different chemical structures, such as oligosaccharides, polyphenols, and even polyunsaturated fatty

acids that have been converted to their respective conjugated forms. Prebiotics have protective effects on the gastrointestinal system by positively modulating gut bacteria. Additionally, they can reduce blood lipid levels, improve insulin resistance, and enhance mineral bioavailability (Davani-Davari et al., 2019; Faustino et al., 2023; Gibson et al., 2017).

3.2.1. Date juice prebiotic activity

Figs. 1 and 2 illustrate the growth curves of the six lactobacilli strains and the five *Bifidobacterium* strains in the different MRS-supplemented media, respectively, while Table 2 lists the specific growth rates for each strain in each growth medium containing different carbon sources. Positive and negative control experiments were conducted to validate the experimental setup. Positive control assays utilized modified MRS broth supplemented with glucose and fructooligosaccharides (FOS) as carbon sources. Conversely, negative control experiments employed MRS broth devoid of any carbon source. As anticipated, the negative control medium (MRS without glucose) exhibited significantly restricted microbial growth for all the strains assayed (low specific growth rates, Table 2), since there is no sufficient carbon source available to allow normal growth of lactobacilli and *Bifidobacterium* strains.

The examination of Fig. 1 and Table 2 showed variations in the growth patterns of the six lactobacilli strains based on the carbon source under study. All strains exhibited good growth in glucose, although at different rates, with L. *plantarum* 299v (0.833 h^{-1}) showing the best growth performance, followed by L. *rhamnosus* R11 (0.752 h^{-1}), L. *paracasei* L26 (0.611 h^{-1}) and L. *casei* 01 (0.601 h^{-1}) (Table 2). Conversely, L. *acidophilus* La5 and L. *acidophilus* Ki reported lower specific growth rates (0.423 h^{-1} and 0.349 h^{-1}, respectively). In general, the maximum absorbance values followed this trend, the L. *acidophilus* strains reached OD_{600nm} levels of 0.9 and 1.4 for L. *acidophilus* Ki and L. *acidophilus* La5, respectively, compared to OD_{600nm} levels of 2.0–2.1 for the other lactobacilli strains studied (Fig. 1). The addition of FOS as the sole carbon source did not enhance the growth of the six lactobacilli strains as much as glucose; specific growth rates were either similar (as reported for the two L. *acidophilus* strains; $p > 0.05$) or 0.10–0.25 units lower ($p < 0.05$; Table 2). The maximum OD_{600nm} levels followed the same trend. Date juice, especially at a concentration of 10 % (w/v), demonstrated a more potent growth-promoting effect compared to FOS and was comparable to the effect of glucose on the growth of all lactobacilli strains examined except for the two L. *acidophilus* Ki and La5 strains. In this latter case growth parameters were improved both in terms of specific growth rates and OD_{600nm} levels. Significant variances between the two concentrations of date juice were noted ($p < 0.05$), particularly in terms of maximum OD_{600nm} levels, with the most notable differences observed in the two L. *acidophilus* Ki and La5 strains. Nonetheless, it is important to emphasize that incorporating media with 2 % date juice led to increased growth rates compared to using FOS for all tested strains.

Based on the above, it can be inferred that in order to achieve optimal growth of lactobacilli species, 10% date juice supplementation is recommended. The higher the percentage of date juice, the higher the concentration of available carbohydrate sources, namely sugars. A higher concentration of available sugars leads to improved growth rates

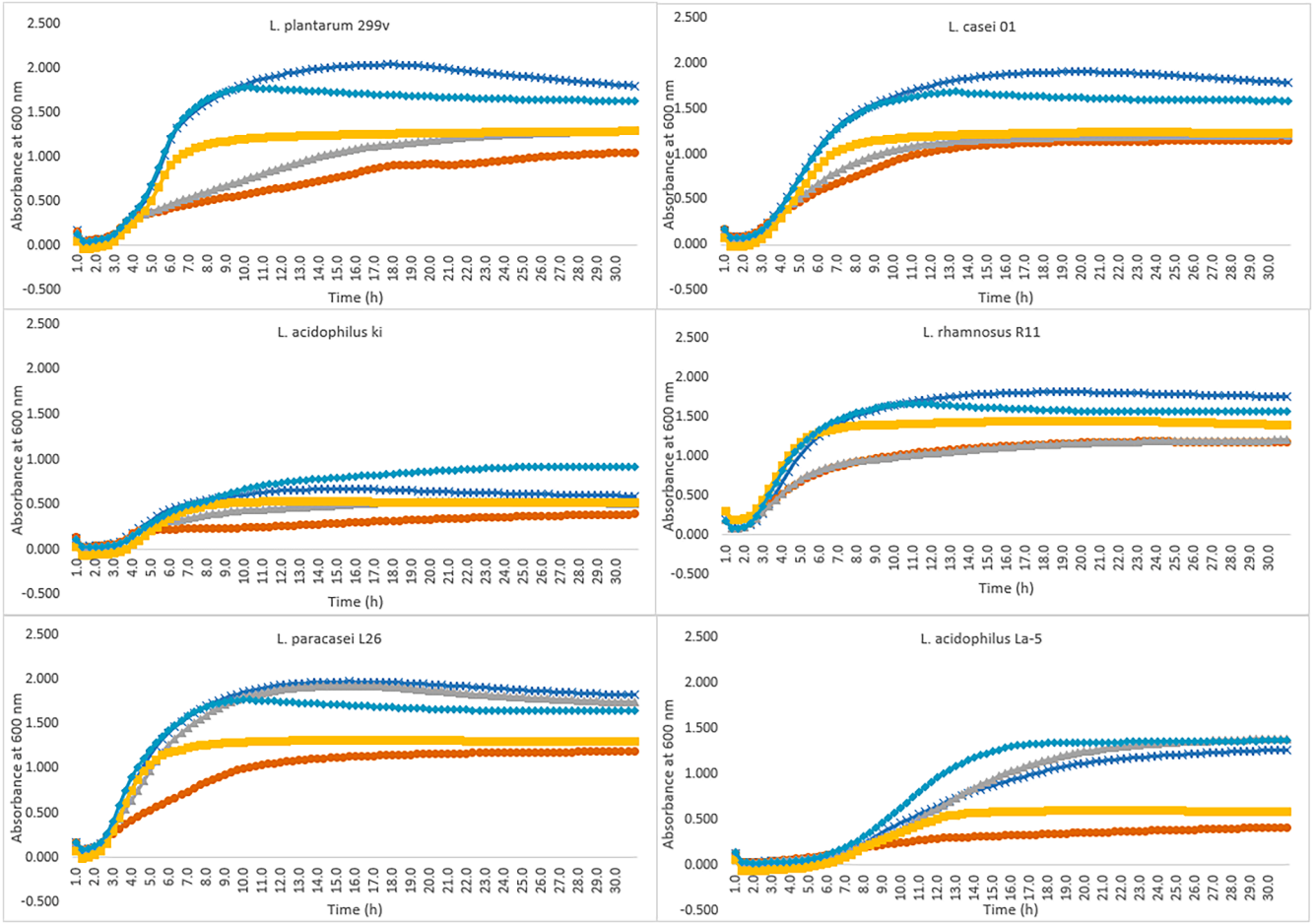


Fig. 1. Growth curves for Lactobacillus strains (L. *plantarun 299v,* L. *acidophilus ki,* L. *acidophilus La-5,* L. *paracasei L26,* L. *casei 01, and* L. *rhamnosus R11)* grown in different culture media [basal MRS medium without glucose (orange), MRS with glucose (dark blue), MRS with FOS (gray) MRS with 2% date juice (yellow) and MRS with 10% date juice (light blue) determined by measuring OD of the cultures at 600 nm over a 30 h-period.

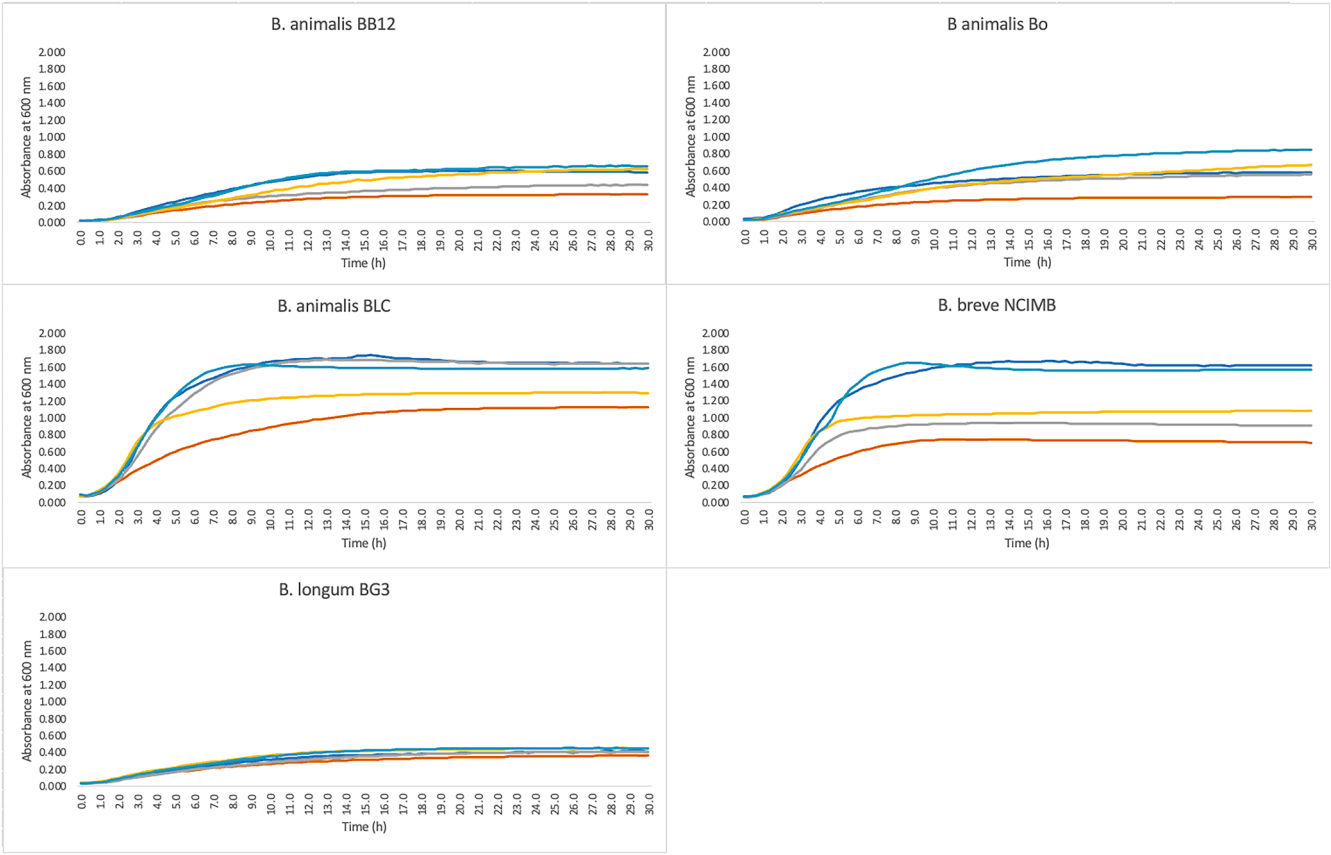


Fig. 2. Growth curves for Bacillus strains (*B. animalis BB12, B. animalis Bo, B. animalis BLC, B. breve NCIMB and B. longum BG3)* grown in different culture media [basal MRS medium without glucose (orange), MRS with glucose (dark blue), MRS with FOS (gray) MRS with 2% date juice (yellow) and MRS with 10% date juice (light blue) determined by measuring OD of the cultures at 600 nm over a 30 h-period.

Table 2
Specific growth rates for the lactobacilli and *Bifidobacterium* strains tested in the different MRS media, with different carbon sources including different date juice (DJ) concentrations (2 and 10 %).

Strain	Specific growth rate (h^{-1})				
	Carbon Source %(w/v)				
	Glucose	None	FOS	DJ 2%	DJ 10%
Lactobacilli					
L. *acidophilus* Ki	0.423	0.265	0.400	0.558	0.480
L. *acidophilus* La5	0.349	0.203	0.307	0.404	0.437
L. *rhamnosus* R11	0.752	0.420	0.568	0.607	0.752
L. *paracasei* L26	0.611	0.339	0.543	0.664	0.650
L. *casei* 01	0.601	0.391	0.515	0.621	0.627
L. *plantarum* 299v	0.833	0.447	0.556	0.754	0.838
Bifidobacterium					
B. animalis BB12	0.596	0.483	0.572	0.523	0.568
B. animalis Bo	0.421	0.403	0.441	0.324	0.394
B. animalis BLC	0.715	0.578	0.604	0.647	0.690
B. breve NCIMB	0.679	0.419	0.616	0.631	0.655
B. longum BG3	0.446	0.429	0.456	0.466	0.472

for bacterial species. Nevertheless, it is worth noting that exceeding the 10 % threshold of date juice may not necessarily result in higher growth rates, as excessively high sugar concentrations could inhibit bacterial growth (Cai et al., 2021; Mizzi et al., 2020).

The growth patterns of *Bifidobacterium* strains were found to be different depending on the carbon source being studied (Table 2; Fig. 2). *Bifidobacterium animalis* BLC exhibited the highest specific growth rates, regardless of the carbon source, while *B. animalis* Bo showed the lowest values (Table 2). This observation was supported by the maximum OD_{600nm} levels reached – 1.8 for *B. animalis* BLC and 0.6 for *B. animalis* Bo (Fig. 2). *Bifidobacterium longum* BG3 also displayed low specific growth rates, which were consistent across different carbon sources; its growth was equally influenced by date juice, glucose, and FOS (the latter being positive controls). With the exception of *B. animalis* Bo, all *Bifidobacterium* strains exhibited similar maximum OD_{600nm} levels when grown in MRS supplemented with 10 % (w/v) date juice compared to glucose. Additionally, growth in MRS supplemented with 2 % (w/v) date juice closely resembled that achieved with FOS for all strains except *B. animalis* Bo.

As seen in Section 3.1, date juice consists mainly of water-soluble solids that are transferred during the production of the co-product. These solids are mainly soluble sugars such as glucose and fructose, which represent 38.2–44.7 % and 36.8–40.1 % of the total dry mass, respectively (Muñoz-Bas et al., 2024; Kamal-Eldin et al., 2020). Supplementing the basal medium with 10% date juice may result in a higher concentration of sugars in the medium compared to the control basal medium containing 20 g/L. The higher sugar concentration will consequently result in higher bacterial growth rates.

3.2.2. Date powder prebiotic activity

Fig. 3 displays the growth curves of the four selected probiotic bacteria (*Lacticaseibacillus rhamnosus* 11, *Lactobacillus casei* 01, *Bifidobacterium breve* NCIMB, and *Bifidobacterium animalis* BLC) to assess the growth-factor potential of date powder at two different concentrations (2 and 6 % (w/v)) over a 24 h period as determined by viable cell enumeration.

By analyzing Fig. 3, it can be inferred that the use of 2 % (w/v) date powder exhibited a prebiotic potential. The media supplemented with date powder (2 % (w/v)) had higher viable cell numbers (CFU/mL)

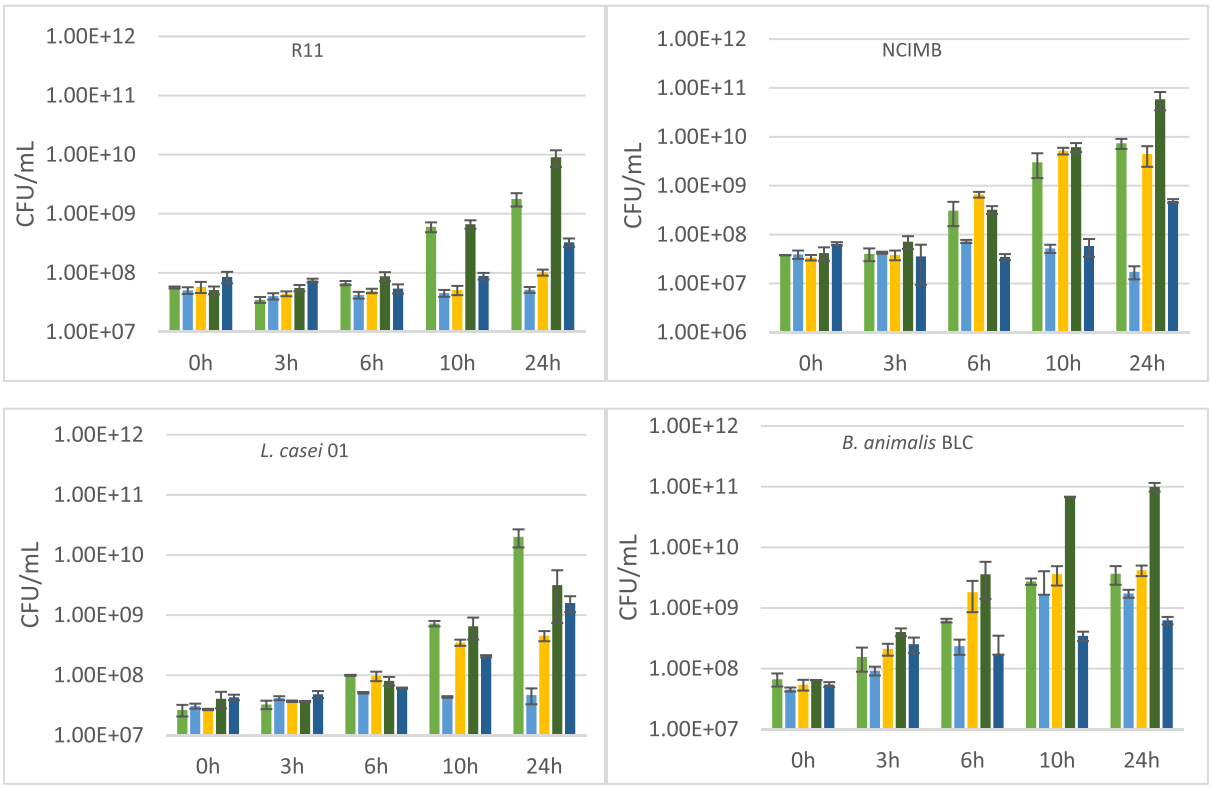


Fig. 3. Evaluation of the growth of selected probiotic bacteria *(Lacticaseibacillus rhamnosus 11, Bifidobacterium breve NCIMB, Lactobacillus casei 01, Bifidobacterium animalis BLC)* in different culture media (light green: MRS with glucose; light blue: MRS without glucose; yellow: MRS with FOS; dark green: MRS with 2% date powder; dark blue: MRS with 6% date powder).

compared to MRS-FOS (prebiotic positive control) and MRS with glucose (positive control) after 24 h of incubation for all the probiotic bacteria tested except for L. *casei* 01. It is noteworthy that the growth promotion of L. *casei* 01 was comparable between the two media supplemented with date powder (2 % and 6 % (w/v)) and even higher than that registered for MRS-FOS. However, when this strain was incubated in MRS with glucose, the highest viable cell numbers were achieved at 24 h. The *Bifidobacterium* strains (*B. breve* NCIMB and *B. animalis* BLC) showed higher viable cell numbers (5.50×10^{10} CFU/mL and 9.77×10^{10} CFU/mL, respectively) than L. *casei* 01 or L. *rhamnosus* 11 after a 24 h incubation period with 2% date powder. When compared to the positive control FOS, the same strains did not reach such high numbers, with viable cell counts of 1.10 and 1.37 log cycles lower at the same 24 h time point, respectively. Notably, the L. *rhamnosus* R11 strain exhibited a particularly pronounced difference, reaching 1.95 log cycles higher when date powder was used.

Typically, food sources of prebiotic compounds contain high levels of carbohydrates, specifically non-digestible carbohydrates such as dietary fibers. As observed in Section 3.1., date powder is a valuable source of dietary fibers, with a TDF threshold of 66.0 ± 1.4 g/100 g. By having high amounts of TDF, date powder is a potential natural source of prebiotic compounds. In light of the aforementioned considerations, it was anticipated that the experimental conditions containing 2 % and 6 % (w/v) date powder would facilitate the growth of the selected probiotic strains. A 2017 study, which explored the prebiotic potential of date seeds on stimulating the growth of lactobacilli strains, yielded comparable results. The presence of date seeds stimulated the growth of lactobacilli strains with values comparable to those observed in the positive controls (Al-Thubiani and Khan, 2017). It is important to note that the prebiotic effect of the developed date powder may result from the combination of fermentable water-soluble simple carbohydrates and dietary fibers.

It should be emphasized that the condition containing 6 % (w/v) date powder yielded lower CFU/mL values compared to 2 % (w/v) date powder, suggesting that increasing the concentration of date powder may not necessarily improve the prebiotic potential. A similar trend was observed in the evaluation of yacon tuber flour's prebiotic potential; the addition of 1 % (w/v) yacon flour revealed better growth outcomes compared to the addition of 2 % (w/v) (Sousa et al., 2015). Therefore, based on the abovementioned findings, 2 % (w/v) date powder appears to be an optimal concentration for promoting the growth of the selected probiotic strains. Furthermore, Fig. 4 corroborates these results by illustrating the temporal evolution of pH during the growth of the four selected probiotic bacteria over a 24 h incubation period. As anticipated, pH levels exhibited a decline over the assessment period, indicating fermentative activity by the probiotic bacteria. This decline can be attributed to the conversion of available sugars into corresponding acids through fermentative activity; growth and acidification activity were well correlated.

In light of the aforementioned considerations, it can be reasonably inferred that 2 % (w/v) date powder exerts a prebiotic effect on the selected probiotic strains.

3.3. Total phenol content and antioxidant activity

Phenolic compounds, the most abundant secondary metabolites in plants, are characterized by a common chemical structure consisting of an aromatic ring with one or more hydroxyl substituents. These compounds are highly effective in neutralising free radicals and exhibit antioxidant properties. Furthermore, phenolic compounds offer a range of health benefits including antibacterial, antihyperlipidemic, anticancer, antioxidant, cardioprotective, neuroprotective, and antidiabetic properties (Al Manary, 2022; Khoddami et al., 2013; Lin et al., 2016). There is a strong correlation between the phenolic compound content and antioxidant activity, meaning that higher levels of phenolic compounds are linked to greater antioxidant activity (Muflihah et al., 2021).

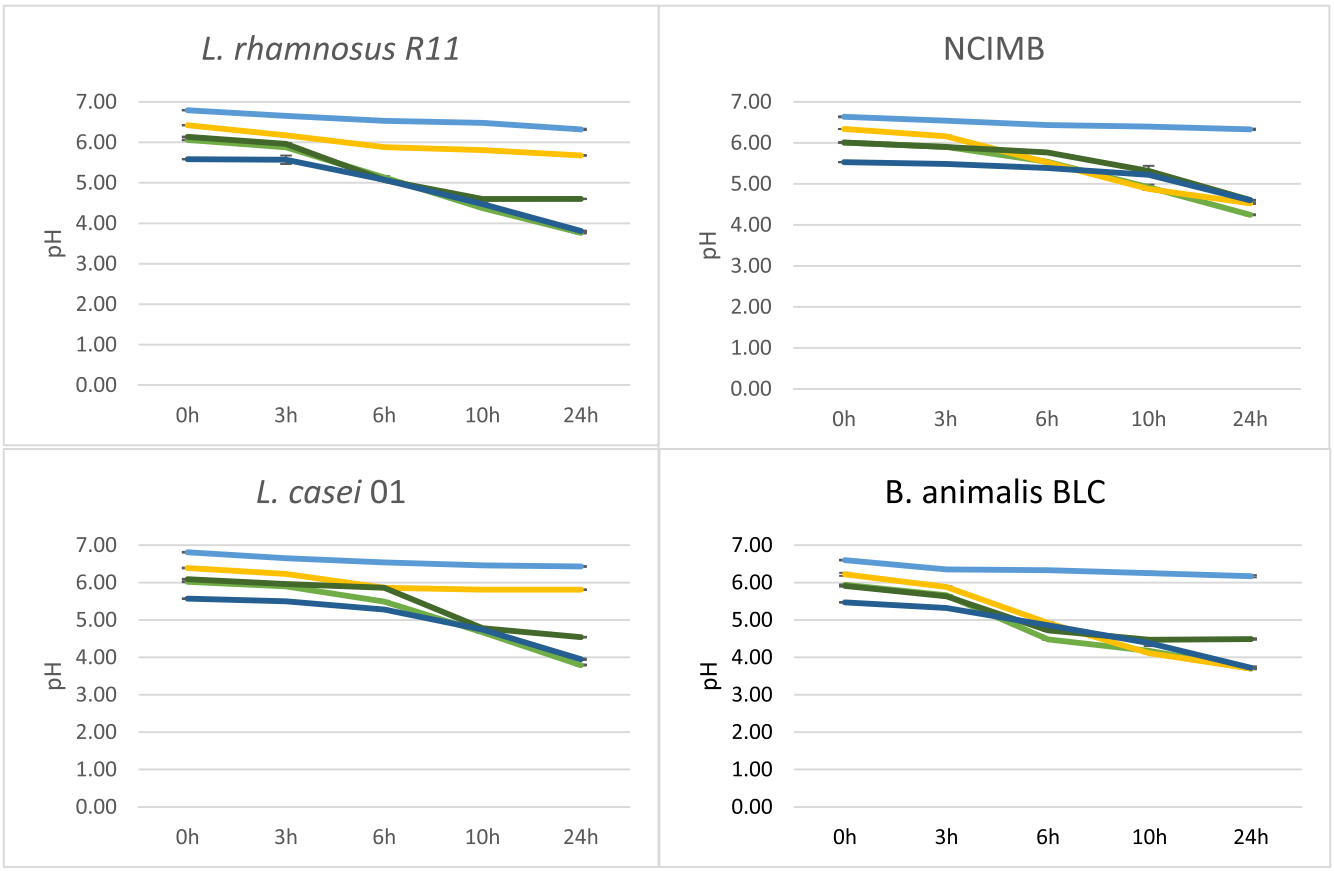


Fig. 4. pH evolution during the growth of selected probiotic bacteria *(Lacticaseibacillus rhamnosus 11, Bifidobacterium breve NCIMB, Lactobacillus casei 01, Bifidobacterium animalis BLC)* in different culture media (light green: MRS with glucose; light blue: MRS without glucose; yellow: MRS with FOS; dark green: MRS with 2% date powder; dark blue: MRS with 6% date powder).

Table 3 shows the total polyphenol content (TPC) and the antioxidant activity of date juice and date powder. As anticipated, date powder (1.575 ± 0.028 mg GAE/g) exhibited higher TPC than date juice (0.146 ± 0.004 mg GAE/mL), resulting consequently in higher antioxidant activity (Table 3). The water extraction process involves the filtration of solid substances from the source water, resulting in the production of the final product. In this context, it is possible that phenolic compounds may remain bound to dietary fibre, resulting in a lower concentration of these compounds in the juice justifying the lower TPC content (Zhang et al., 2017). As previously stated, a correlation has been identified between TPC and antioxidant activity; an increase in TPC is accompanied by an increase in antioxidant activity, and vice versa (Kumar et al., 2014; Muflihah et al., 2021). In this context, date juice exhibited a relatively low antioxidant activity (702.060 ± 23.147 μmol of TROLOX equivalent/mL), which correlated with the low phenolic compounds content present, whereas date powder reported a high antioxidant activity (5823.026 ± 27.247 μmol of TROLOX equivalent/g) aligned with the higher TPC content (Table 3). The high antioxidant activity observed in the present study is in line with the findings of previous research, which has reported the potential antioxidant activity of date fruits (Allaith 2019; Rahmani et al., 2014). Date fruits are considered to be good sources of phenolic compounds (Alsuhaymi et al., 2023). Higher concentrations of phenolic compounds are mainly found in the pulp and in the seed fraction of the date fruit (Khatib et al., 2022). Date powder studied herein is mainly composed of grinded and dried fresh date pulp, resulting therefore in the high concentration of TPC observed in Table 3, namely 1.575 ± 0.028 mg GAE/g TPC, in date fruits ranges from 2.06 ± 0.06 to 6.53 ± 0.18 mg GAE/100 g DW (Ali Haimoud et al., 2016) to 753.30 mg GAE/100 g DW (Shahdadi et al., 2015), depending on several factors such as cultivar, growth conditions, ripening stage and extraction procedure among others (AlFaris et al., 2021; Bouhlali et al., 2017). A comparison of the obtained results with those reported in the literature, considering the final ripening stage, indicates that the date powder contains approximately one-third of the TPC found in the whole date fruit. Consequently, it can be inferred that approximately one-third of the TPC found in the date fruit is associated with the dietary fiber present in the date pulp.

Furthermore, it is important to note that the same factors responsible for the amount of TPC found in the date fruit are also responsible for the relative content of the different polyphenols found in date fruits, such as cinnamic and coumaric acids and their derivatives, including ferulic, sinapic, syringic, vanillic, gallic, caffeic, protocatechuic and dactilyferic acids, as well as flavonoid glycosides (luteolin, methyl luteolin, quercetin, and methyl quercetin), flavones, flavanols (catechin, epicatechin), flavaxanthin and anthocyanins (Jdaini et al., 2023).

Table 3
Total Phenolic content (TPC), antioxidant activity (ABTS assay), antidiabetic activity (α-glucosidase inhibition) and antihypertensive activity (inhibition of angiotensin-I-converting enzyme (ACE)) of date juice and date powder.

	Date juice	Date powder
TPC (mg GAE/X)	0.146 ± 0.004b (mg GAE/mL)	1.575 ± 0.028a (mg GAE/g)
ABTS	702.060 ± 23.147b (μmol of TROLOX equivalent/mL)	5823.026 ± 27.247a (μmol of TROLOX equivalent/g)
α-glucosidase inhibition (%)	76.34 ± 0.78b	87.62 ± 2.04a
ACE inhibition (%)	55.73 ± 4.92b	92.24 ± 0.73a

$n = 3$. a-b: Different letters in the same row indicates significant differences ($p < 0.05$).

3.4. Antidiabetic activity

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, which can lead to long-term damage to various organs in the body. According to recent data from the World Health Organization (WHO) (2024), approximately 422 million individuals worldwide are living with diabetes, with a significant proportion residing in low- and middle-income countries. Furthermore, this condition is directly responsible for an estimated 1.5 million deaths annually, underscoring its substantial impact on global health (World Health Organization, 2024).

The α-glycosidase enzyme resides in the intestinal brush border. It transforms oligosaccharides and disaccharides into monosaccharides, facilitating carbohydrate absorption and elevating blood sugar levels. α-glycosidase inhibitors can postpone carbohydrate absorption via competitive inhibition, impeding the hydrolysis of disaccharides and glucose absorption (Vichayanrat et al., 2002). The search for natural sources of α-glycosidase inhibitors including those sourced from fruits and vegetables, has attracted the attention of the scientific community. Several authors have reported the antidiabetic properties of fruit and vegetable extracts, including kiwi, lemon pulp, lemon peel, pear red onion and tomato, among others (Wu et al., 2015). In most of these cases, the antidiabetic activity was positively linked to the polyphenol content.

Regarding the date co-products under analysis, Table 3 shows that the date powder (87.62 ± 2.04 %) exhibited greater ($p < 0.05$) α-glucosidase inhibitory activity than date juice (76.34 ± 0.78%). This result was expected because date powder is richer in phenolic compounds compared to date juice (see Section 3.3), and therefore a greater α-glucosidase inhibitory activity was anticipated. Although date juice has fewer phenolic compounds (0.146 ± 0.004 mg GAE/mL), its α-glucosidase inhibitory activity is relatively high, namely 76.34 ± 0.78 %. The hypothesis is that the date fruit may also contain water-soluble non-phenolic bioactive compounds that have activity against the α-glucosidase enzyme (Khan et al., 2016; Mia et al., 2020). Similar results were reported by El Abed et al., (2017). Furthermore, a 2017 study demonstrated how various extracts from date fruits have α-glucosidase inhibitory activity, correlating the obtained results. El Abed et al. (2017) observed a strong inhibitory activity against α-glucosidase in an aqueous ethanolic extract from date fruits, which was even higher than that against α-amylase.

Furthermore, the evidence suggests that date fruit has *in vivo* antidiabetic effects. *In vivo* reports have demonstrated that short-term administration (one month intake) results in reduced blood glucose levels and increased insulin concentration through mechanisms such as an increase in the number of β-cells, stimulation of insulin secretion, and lowering of gastric emptying by the action of polyphenol (Evans et al., 2018; Mia et al., 2020).

The antidiabetic assay performed solely determines the inhibitory activity of α-glucosidase. Therefore, further *in vivo* studies are suggested to fully understand the antidiabetic capacity of both date juice and powder.

3.5. Antihypertensive activity

Hypertension is a leading risk factor for cardiovascular disease, causing widespread morbidity and mortality globally. According to WHO, one in three adults worldwide is affected by hypertension (World Health Organization, 2023). Additionally, hypertension is approximately twice as prevalent in individuals with diabetes compared to those without diabetes, making it a significant public health concern (Farida et al., 2023).

Suppressing angiotensin-I-converting enzyme (ACE) is a crucial strategy for managing hypertension, a global health crisis of epidemic scale and a significant contributor to cardiovascular disease risk (Faustino et al., 2023; World Health Organization, 2023). Lifestyle changes, including diet, have recently attracted interest due to the undesirable side effects associated with synthetic ACE-inhibitors. Consumption of fruits and vegetables, which are rich in vitamins, minerals, and bioactive compounds, has been proposed as a relevant prevention factor. Furthermore, fruits and vegetables are being investigated as potential sources of natural compounds with antihypertensive properties, which is of great interest (Yousefi et al., 2021).

Date fruits contain various bioactive compounds with potential health benefits, including antioxidant, antimicrobial, anticancer, and anti-inflammatory properties (Muñoz-Tebar et al., 2023). The antihypertensive activity of date fruits may be mainly due to the presence of flavonoids, minerals, vitamins, and fibers (Yousefi et al., 2021). Table 3 displays the ACE inhibition percentages of date juice and date powder. It is worth noting that date powder has a high ACE inhibition percentage, close to 100% (92.24 ± 0.73 %), while date juice presents an ACE inhibitory activity of 55.73 ± 4.92 %. Date fruits contain several bioactive compounds that regulate and manage hypertension, including lauric acid, linolenic acid, palmitic acid, tocopherols, β-sitosterol, and isosorbide (Obode et al., 2020). Date powder has a higher inhibition of ACE than date juice because the bioactive compounds are mainly found in the flesh, not always water soluble and therefore not transferable to date juice. However, it is important to note that date juice still has a good ACE inhibitory activity of over 50%, indicating the presence of water-soluble ACE inhibitory compounds.

Several reports have shown that date fruits have a potent antihypertensive effect due to their ability to inhibit angiotensin-converting enzyme (Al-Dashti et al., 2021; Fernández-López et al., 2022; Vayalil, 2012). This ability is mainly attributed to certain polysaccharides present in date fruits. Additionally, date fruit is rich in potassium and low in sodium, which helps to maintain electrolyte balance and further contributes to controlling blood pressure.

The valorization of co-products from the fresh date processing industry has made it possible to obtain two high-added value products with a chemical composition that is very interesting for the food industry, in an eco-efficient way and boosting the circular economy of palm-growing ecosystems. If this is complemented with their *in vitro* demonstration of important functional properties such as antioxidant, prebiotic, antihypertensive and antidiabetic activities, it will allow their application in the development of potentially healthier foods. This opens the door to their industrial processing and marketing as high added-value food ingredients. Thus, the food industry can use them for new food development and innovation projects to meet the demands of today's consumers, i.e. healthier and more sustainable foods.

4. Conclusions

The development of date by-products has made it possible to obtain two value-added food products, namely date powder and date juice. In terms of nutritional evaluation, date powder was found to be richer in nutrients and contained high levels of total dietary fiber, while date juice had high concentrations of water-soluble sugars. In this sense, date powder provided higher levels of total phenolic compounds and *in vitro* bioactivities such as antioxidant, antidiabetic and antihypertensive activities. Date juice, although poor in various nutrients, still showed activity in all the assays tested. Furthermore, in terms of prebiotic activity, the sugar-rich date juice showed a positive role for all probiotic strains tested at both 2% and 10% (w/v). Date powder also showed prebiotic potential due to its high concentration of dietary fiber. However, it is important to note that date powder in high concentrations (6% (w/v)) could inhibit probiotic growth. In this sense, this study is the first to analyse the potential bioactivities and prebiotic potential of fresh date by-products.

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Ethical statement

The authors declare that the work described has not involved experimentation on humans or animals.

Data availability

Data are available on request from the corresponding author.

CRediT authorship contribution statement

Clara Muñoz-Bas: Writing – original draft, Methodology, Formal analysis. **Rita Vedor:** Writing – original draft, Methodology, Formal analysis. **Daniela Machado:** Writing – review & editing, Methodology, Formal analysis. **Joana Cristina Barbosa:** Writing – review & editing, Methodology. **Ana Maria Gomes:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization. **José Angel Pérez-Alvarez:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Juana Fernández-Lopez:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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7.4. PUBLICACIÓN 4

Capítulo: *Bioactive compounds of fruits, vegetables and their coproducts in the development of dairy functional products*

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BIOACTIVE COMPOUNDS OF FRUITS, VEGETABLES AND THEIR COPRODUCTS IN THE DEVELOPMENT OF DAIRY FUNCTIONAL PRODUCTS

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Abstract

Milk and dairy products have been widely studied as food matrices for the development of functional foods mainly due to their high nutritional quality and potential health benefits, great consumers' acceptance and wide technological versatility. In addition, fruits, vegetables (in several forms including fresh, juices, powder, puree, and extract) and their coproducts are important sources of bioactive compounds (dietary fiber, polyphenols, carotenoids, natural colorants, minerals, vitamins, phytoestrogens, and anthocyanins, among others) which are receiving increased attention for their potential health benefits. Therefore, the development of functional dairy products by the addition of some of these bioactive compounds is currently a very promising research line for the dairy industry. The technological strategy used for their incorporation into the different dairy matrices (yogurts, cheeses, ice creams, dairy desserts, etc.) and their effect on the nutritional improvement of the product, the stability of the incorporated bioactive compounds and the sensory acceptance of the final dairy product will be discussed.

Keywords: Bioactive compounds, Cheeses, Dairy desserts, Ice creams, Reformulation, Yogurts

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1. Fruits, vegetables and their coproducts as source of bioactive compounds

Fruits and vegetables are excellent sources of bioactive compounds mainly dietary fiber and phytochemical compounds (flavonoids, carotenoids, anthocyanins, and phenolic acids, among others) which have positive actions in the body promoting good health (Coman et al., 2020). The positive actions associated with phytochemical compounds are due to several proven bioactive properties, mainly antioxidant, antimicrobial, and anti-inflammatory properties (Aqilah et al., 2023), which, in turn, are associated with the control of the development and progression of most chronic diseases such as obesity, diabetes, cardiovascular diseases, neurodegenerative disorders, and cancer (Kainat et al., 2022). Dietary fiber provides also numerous health advantages including lowering the risk of heart disease, obesity, and type 2 diabetes which has been related to the modulation of the gut microbiota (Viuda-Martos et al., 2010; Fu et al., 2022). It has been reported that dietary fibers interact directly with gut microbes leading to the production of key metabolites (such as short-chain fatty acids) which modulate biological processes essential for health. If the healthy balance of the microbes in the gut is disrupted (for example a low intake of dietary fibers and an increasing amount of fat and sugar) there is a greater predisposition to suffer various chronic inflammatory diseases, such as colorectal cancer, intestinal bowel disease, autoimmune diseases, obesity, and other associated pathologies (Makki et al., 2018).

The type and amount of these bioactive compounds found in fruits and vegetables depend on the type of product, variety, growth conditions, etc. In general, it could be said that common fruits (apple, strawberry, blueberry, papaya, and citrus fruits, among others) contain dietary fiber, phenolic acids, flavanols, carotenoids, and anthocyanins, and common vegetables (potato, tomato, carrot, beetroot, and broccoli, among others) mainly contain dietary fiber, carotenoids, betalains, and flavonoids (**Table 1**). As well as the original raw materials (fruits and vegetables), the wide range of co-products generated during their processing (leaves, peels, seeds, stones, pomaces, discarded or low-grade products, etc.) are also considered important sources of these bioactive compounds (Fernández-López et al., 2004; Kainat et al., 2022; Aqilah et al., 2023), which subjected to appropriate extraction techniques could come back to the food chain as valuable ingredients for the development of functional foods (Bas-Bellver et al., 2020). In some cases, these co-products have even higher content of some bioactive compounds than the main products (Sivananth et al., 2017). For example, date seeds have higher dietary fiber content and polyphenol compounds than date pulp (Fernández-López et al., 2022); peels of lemons, oranges, and grapes as well as the seeds of avocados and mangos have 15% more phenolic content than of the fruit pulp (Sivananth et al., 2017); potato peels have higher dietary fiber, phenolic, flavonoid and anthocyanin content than the flesh (1.6, 2.9, and 1.5 times higher, respectively) indicating that these compounds are accumulated in the peel (Ncobela et al., 2017; Makori et al., 2022). Therefore, valorization of wastes and co-products from fruits and vegetable processing not only is a valuable strategy to obtain high-added value compounds but also frames within the Sustainable Development Goals of the United Nations for sustainable development, reducing food waste, and so contributing to the circular economy (Gonçalves & Maximo, 2022).

Regardless of the source, these bioactive compounds can be obtained (isolated or extracted) by applying several extraction techniques, from the conventional extraction process using solvents to other emerging and green technologies such as enzymatic digestion, ultrasound, microwave, ohmic heating, supercritical fluids or electric pulses (Carrillo et al., 2022; Rodríguez-García & Raghavan, 2022), all of them characterized by

their greater sustainability and efficiency. For example, ultrasound-assisted extraction has been reported as an useful method for pectin extraction from the waste peel, pomace, and rind of several fruits (passion fruit, pomegranate, orange, grapefruit, mango, and banana) (de Oliveira et al., 2016; Hosseini et al., 2019, 2020; Aqilah et al., 2023) and vegetables (tomato, eggplant, and sunflower) (Grassino et al., 2016; Ponmurugan et al., 2017; Kazemi et al., 2019). The fermentation of apple peel with *Aspergillus* spp. enhanced the extraction of phenolic acids and flavonoids increasing their antioxidant activity between 3-5 times compared with unfermented apple peels (Gulsunoglu et al., 2020). Microwave-assisted extraction was successfully used for the extraction of phenolic acids, flavonoids and anthocyanins from eggplant peels, reaching greater yields than using conventional extraction technologies (Doulabi et al., 2020). Supercritical fluid extraction has been applied on tomato skin, pomegranate peel, and *Capsicum annuum* industrial waste to extract carotenoids, flavonoids and phenolic acids, increasing total mass yields although in some cases reducing antioxidant activity (Pellicanò et al., 2019; Soldan et al., 2021; Kupnik et al., 2022). Pulse electric field extraction has been successfully applied on onion waste enhancing quercetin extraction, on citrus peels enhancing hesperidin, and narirutin extraction or on tomato waste to enhance carotenoid extraction (Tehrani et al., 2019; Andreou et al., 2020; Hwang et al., 2021). All these bioactive compounds can come back to the food chain as functional ingredients to enrich or fortify the food matrix to which they were added.

2. Development of dairy functional products

Due to the nutritional content and health benefits attributed to the consumption of dairy foods (Verruck et al., 2019), their great consumer acceptance, and the wide versatility of the dairy matrix, the food industry has been a pioneer in the development of functional dairy products. Although the definition of functional food has changed over the years, nowadays the term functional food is used to describe foods that provide health benefits beyond meeting basic nutrition needs due to their physiologically active food components (i.e. bioactive compounds) (Temple, 2022). In addition to the incorporation of the bioactive compound in sufficient quantity to exert its beneficial effect, it is necessary to ensure its stability in the matrix in which it is incorporated, for which different technological strategies can be used, depending on the type of bioactive compound and the type of dairy matrix in which it is going to be added. There is different liquid (milk and some fermented milks), semi-solid (yogurt), or solid (cheese) dairy matrices, in turn, physiochemically they can be emulsions (milk, butter), gels (yogurt, cheese) or foams (ice cream), and so, not only the bioactive compound added but also the selected dairy matrix are decisive in the result obtained. The most studied dairy matrices used in the development of functional products have been yogurt, cheese, ice creams, and some dairy dessert. On the other hand, the most used strategies for the addition of bioactive compounds into these diary matrices are direct addition and encapsulation (Villamil et al., 2020). Although the direct addition of compounds is technologically easier, sometimes can generate undesirable physical, chemical, and sensorial changes decreasing the final product quality (Robertson et al., 2016). For this reason, the encapsulation of these compounds, previously to their addition into the dairy matrix is a very interesting strategy because it should allow both, the compounds protection and the preservation of the sensorial characteristics of the dairy product, achieving a higher overall acceptance. In addition, this incorporation strategy has been shown to increase the compound bioavailability, allowing a greater health benefit for the consumer's health (Tolve et al., 2020).

2.1. Functional yogurt

Yogurt is the most consumed dairy product, resulting for the fermentation of milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It is characterized by a high nutritional value, even higher than other unfermented dairy products, due to the increase in vitamin B, conjugated linoleic acid, bioactive peptides, protein digestibility and calcium solubility (Fardet et al., 2018), and also by the beneficial health effects associated to its consumption due to the presence of these viable microorganism (probiotics) (Verruck et al., 2019). In addition, yogurt texture (semi-solid) and viscosity can even enhance some of these properties.

Extracts from vegetables, fruits and their co-products, mostly obtained as a powder after dehydration and grinding of the raw material and so, with a high content in dietary fiber and other bioactive compounds (mainly polyphenols), have been directly added to yogurt elaboration process (Table 2) with different purposes: as stabilizer or texturant, and/or as antioxidants, and/or as source of fiber to fulfill dietary needs, increasing satiety and modulating appetite. Regarding the use of extracts from fruits, Srimali et al. (2019) added citrus fiber to drinking yogurt (0.1-0.3%) as stabilizer in substitution of gelatin and carrageenan, without modification of desired physicochemical and sensory properties. The addition of 1% of orange fiber (from orange co-products) to yogurt reduced the syneresis, improved the creaminess sensory scores, increasing gel firmness, and stickiness compared to control yogurt (García-Pérez et al., 2006). Fiber-rich extracts from both banana and banana peel, in percentages from 0% to 1% were added to yogurts by Safdari et al. (2021). As a result, they obtained that the sample with 1% banana fiber showed better results taking into account the amount of fiber obtained in the final product, as well as microbiological counts and physicochemical properties (improved syneresis, pH, viscosity and interfacial tension). Extracts from the skin of dragon fruit were added to frozen yogurts (0-45%) with the aim of increasing their nutritional value (Analianasari & Apriyani, 2018). They reported that the greater the amount of extract added, the greater the antioxidant capacity, the amount of fiber and the total acidity, corresponding the best results with the highest concentration used. Several authors have studied the effect of the addition of extracts from vegetables and legumes to yogurts. For example, the use of carrot extracts (0-5%) resulted in yogurts with high bioactive and functional potential due, especially, to the presence of β-carotene (Šeregelj et al., 2021). Lentil flour has been added to yogurts (0-4%) improving their physicochemical and structural quality (better results at higher concentrations) and adding functional properties (prebiotic activity) (Hag et al., 2019; Benmeziane et al., 2021). Extracts from wild and aromatic herbs have also been studied as source of bioactive compounds in yogurt production. For example, Dabija et al. (2018) used extracts of four different wild herbs (thistle (*Silybum marianum L.*), hawthorn (*Crataegus monogyna*), sage (*Salvia officinalis* L.) and marjoram (*Origanum vulgare L.*) in amounts up to 1%. After analysis, it was shown that the addition of 0.5% thistle extract improved the physicochemical characteristics and the water holding capacity of yogurts. While the addition of 1% marjoram extract showed better results in terms of the nutritional value of the yogurts, as well as better results in terms of antioxidant properties. Sahingil and Hayaloglu (2022) studied the effect of the addition of extract from wild rosehip shrub (*Rosa canina L.*) up to 20% on the yogurt quality. They reported that yogurts with the highest amounts of extract (15-20%) showed better functional, nutritional, antioxidant and sensory characteristics due to the fact that the rosehip bush is a great source of phytonutrients, among which phenolics (antioxidant substances), natural sugars and organic acid, are the predominant. Ozturk et al. (2018) used the peeled and unpeeled oleaster (*Elaeagnus angustifolia* L.) flour (1% and 2%) for the enrichment of yogurts obtaining a product with higher phenolic content, antioxidant

activity, water holding capacity, viscosity index and better microstructural characteristics than control. In addition, the fermentation time was reduced and yogurts containing 2% unpeeled oleaster flour showed similar consumer overall preference scores comparing to plain yogurt. Silva-Dantas et al. (2022) added flour from cactus (*Pilosocereus gounellei*) to yogurts (up to 2%), although the best results were obtained when it was added at 1%. The addition of this extract resulted in a yogurt with higher mineral concentration, and antioxidant activity due mainly to the phenolic compounds and flavonoids from the cactus extract, mainly catechin, epigallocatechin gallate, procyanidin A2 and syringic acid.

The use of extracts from cereals for the supplementation of yogurts has also been explored. Wu et al. (2023) added different amounts of rice bran (up to 3%) to yogurts obtaining the best results when it was added at 1%. Rice bran produced a more compact and porous yogurt, with less whey drained, greater stability, thus resulting in a more functional product, which was attributed to the high dietary fiber (including mainly cellulose, hemicellulose, pectin, arabinoxylan, lignin and β-glucan) and phenolic acids content of rice bran. On the other hand, Campos et al. (2018) used rice and oat extracts even as milk substitutes (at 50%) in yogurt production. These authors demonstrated that the substitution of milk by these extracts did not affect the fermentation process, obtaining a viable alternative with better nutritional properties (lower lipid content and higher fiber and protein content than control). In addition, the best acceptability was obtained when the oat extract was used, even higher than the acceptability of normal Greek yogurt. Lately, there are a lot of works about the addition of seeds and their coproducts to yogurts. Chia seeds have aroused great interest for incorporation into yogurts either alone or in combination with other products. Eker & Karakaya (2020) added chia seeds to yogurt (0-4%) to improve its nutritional value. The addition of 4% resulted in yogurts with higher protein content and better fatty acid profile. On the other hand, in the study by Kown et al. (2019) the use of extracts from chia seeds (0.1%) decreased the fermentation time, improved the physicochemical properties and showed antioxidant activity. Kowaleski et al. (2020) studied the effect of the combination of chia seeds and strawberries on yogurt. This addition was carried out by alternating the amounts of chia seeds (0% to 14%) with those of strawberries (8% to 26%). The best acceptability (70%) was obtained combining 6% chia seeds with 12% strawberries. In addition, they demonstrated that the presence of chia seeds in the yogurt increased the levels of crude protein, total lipids, dietary fiber and PUFA, especially omega-3, thus obtaining a yogurt with high nutritional and functional value. Not only chia seeds but also their mucilage have been used in the development of functional yogurts. Ribes et al. (2021) reported that the use of this mucilage (2.5 to 7.5%) in yogurts resulted in reduction of syneresis, better consistency, firmness, viscosity and resistance to stress and also better nutritional value (higher fiber content), without adversely affecting the sensory properties. Other seeds frequently used in yoghurt production are quinoa seeds. Huang et al. (2022) added quinoa seeds mixed with soybeans in order to obtain a more functional yogurt. Yogurts with better proximal composition, water holding capacity, cell viability, color and rheological properties were obtained. In addition, these yogurts showed a high acceptability by consumers. Basiri et al. (2022) added flaxseed without mucilage (3%) to yogurts improving their nutritional value mainly due to their content in polyunsaturated and omega-3 fatty acids, proteins with a high level of arginine and cysteine, dietary fiber and phenolic compounds.

2.2. Functional Cheese

It is known that consumption of cheese, together with other dairy products, at recommended levels, represents health benefits such as improved immune system function, reduced cardiovascular risk, reduced risk of bone loss (Gebreyowhans et al.,

2019; Verruck et al., 2019). Since the last decades, there has been a great interest in the dairy industry to develop healthier products by incorporating natural ingredients as herbs, spices, fruit and vegetable products and by-products to enrich dairy products and enhance their functional properties biological value and flavour (Farahat et al., 2021). Furthermore, the food industry is committed to reduce the use of synthetic ingredients and to provide preference to natural ingredients. Cheese has been reported as an excellent matrix for the release of bioactive compounds, and thereby increasing its functional benefits. Processed cheese structure and properties allow for the inclusion of bioactive substances and functional ingredients.

Table 3 shows some studies focused on the enrichment and improvement of cheeses by the incorporation of different natural ingredients source of nutrients, vitamins, fibers and bioactive compounds, as mushroom, broccoli, carrot, pepper, tomato, and others. Processed cheese has been presented as a suitable carrier for the incorporation of based-plant ingredients that enhance nutritional, functional and/or technological properties (Basuony et al. 2022). Processed cheese is elaborated by blending cheese of different ages and degrees of maturity, which promotes the incorporation of novel natural ingredients (vegetables like mushrooms, spices, herbs, and fruits, etc.) (Table 3) During cheese processing, it is important to avoid that these natural ingredients may be affected (Khider at al., 2017) and sometimes it is necessary to protect their bioactive compounds.

Broccoli (*Brassica oleracea*) has been incorporated in some cheeses by its high phytochemical content. This green vegetable is one of the plants with the highest content of the health promoters (as vitamins, dietary fiber, phenolics and sulfur compounds, and others), which would be protecting against lipid oxidation, cancer, arthritis and heart disease (Sharma et al., 2011; Abd El-Montaleb et al., 2022). It has been reported the broccoli paste had a stimulatory effect on the growth and activity of bacterial strains (Awad et al., 2012), and could explain the increase on the development of acidity during storage periods of Labneh cheese (Abd El-Montaleb et al., 2022). Some authors have reported that broccoli had an impact on the texture of soft cheese due to its high fiber content, which could be increasing the water retention capacity, thereby increasing the hardness and decreasing the cohesiveness, gumminess, chewiness and elasticity of the dairy product (Awad et al., 2012; Abd El-Montaleb et al., 2022). Another sensory impact reported is the presence of green color, which affects product acceptance and decreased quality attributes of cheese with added vegetables with the storage period (Abd El-Montaleb et al., 2022). Other green vegetable widely reported as a functional ingredient is the spinach, which present a great nutritional and healthy composition of vitamins and minerals, photochemical and bioactive ingredients. El-Sayed (2020) reported a novel functional cheese with spinach nano-powder (0.5, 1 and 2%) to improve the nutritional values. This novel dairy product presented better nutrients (higher fiber, protein, mineral and total phenolic content) and antioxidant activity, moreover those containing 0.5% and 1% spinach presented higher values for sensory parameters than other treatments. Other study added *Spirulina* (*Spirulina maxima)* powder (1, 2 and 3%) to obtained a spreadable processed cheese with a special color (green), good physical properties, high nutritional value, antioxidant activity and sensorial scores, concluded processed cheeses with 1 or 2% *S. maxima* were more acceptable than those of 3% (Mohamed et al., 2020). Carrot (*Daucus carota* L.) is important source of micronutrient, as vitamin A and minerals (Salehi, 2021), has been reported to increase the oxidative and color stabilities of cheeses (Bandyopadhyay et al., 2008) and has been used for analog processed cheese fortification (Mohamed et al., 2016). Basuony et al. (2022) studied the suitable strategy for the incorporation of beta-carotene from carrot co-products to improve the bioactive compound content and functionality of processed cheese. They report that encapsulation

of β-carotene could successfully help to provide carotene to be more resistant to pasteurization or sterilization treatments and to improve the nutritional value, sensory acceptability, and economic advantages of processed cheese than β-carotene.

Tomato is source of bioactive compound, as lycopene which presented antioxidant activated and health benefits, and it could be used to enhance food ingredients. Nevertheless, lycopene can be affected by the ripening process of cheese by oxidation and isomerization (Jeong et al., 2017) and the microencapsulation could protect this bioactive compound during food processing. Jeong et al. (2017) improved lycopene content of 'Queso Blanco cheese' which was supplemented with lycopene-enriched tomato extract microcapsules, it would show that microencapsulation stabilities bioactive compounds. Supplemented 'Queso Blanco cheese' presented lower Lactic acid bacteria counts in 'Queso Blanco cheese' because of the antibacterial activity of lycopene. Also, the supplementation of powdered microcapsules containing tomato extracts improved the texture of 'Queso Blanco cheese', due to the reduction in pH and increases hydrophobic interactions between protein molecules, induced a final product harder and more elastic (Jeong et al., 2017).

Other authors added tomato juice in spread cheese obtained a good and acceptable cheese with high nutritional and healthy food and they concluded that lycopene from tomato juice provided red color attractive which may be a claim for children that encourages the consumption of functional products with antioxidants and health effect (Mehanna et al., 2017). In this approach to enhance nutritional value and consumer acceptability, Atwaa et al. (2020) elaborated spreadable processed cheese supplemented with red pepper (*Capsicum annuum* L.) paste (10% or 20%) which presented higher antioxidant content, low sodium and potassium content, and high levels of minerals., The cheese with 20% sweet red pepper obtained the highest score for appearance and healthy functional properties. Mushroom has been other vegetables products have been used to obtained functional processed cheese by different authors. Petrovic et al. (2015) reported a cream cheese enriched with 3% dry powder of chestnut mushroom (*Agrocybe aegerita* (Brig.) Sing), which presented a higher nutritional value and highly accepted by panellists. Khider at al. (2017) processed cheese with fresh mushroom (*Pleurotus ostreatus*) (at levels of 0, 5, 10 and 15%) or mushroom powder (at levels of 0, 1, 1.5 and 2%) to improve its nutritional composition, microbiological quality and functional and sensory properties. They showed that adding mushroom powder improved flavor and texture, which may be related to the polysaccharides and increased protein/DM ratio and ash content. El-Loly et al. (2021) incorporated date syrup at 15, 20 and 25% to obtain a healthy natural cheese to which present an improvement in Na/K ratio and a good sensory score and overall acceptability. The decrease in Na/K ratio also has been observed by other authors (El-Loly et al., 2022). Lucera et al. (2018) evaluated the effect of the added of different by-products, as broccoli, artichoke, tomato peel, red and white grape pomaces by-products, and corn bran at a concentration of 5%, on physicochemical and sensory quality of spreadable cheese. The best results were obtained by samples containing grape pomace, which obtained higher total phenolic content, flavonoids, and antioxidant activity followed by broccoli, artichoke, corn bran, and tomato peel by-products, compared to the control cheese.

Other authors proposed to improve nutritional values of flavored-processed cheese incorporated a vegetables mixture (mushrooms, dill, leeks, parsley, celery, green peas, green beans, squash, potatoes, and carrots) with ratios at 2.5, 5, 7.5, and 10%. They obtained a novel cheese with improvements in nutritional composition, physicochemical properties and rheological and sensory characteristics (El-Loly et al., 2022). The cheese with vegetables mixture presented a higher content in poly-unsaturated fatty acids, which

may be due to linoleic acid from the mushroom, and vitamin A, which may due to converted some carotenoids found in plants (α-carotene, β-carotene, and β-cryptoxanthine in vitamin A (El-Loly et al., 2022). In this same way, Farahat et al. (2021) formulated processed cheese enriched with dried vegetables combination (mushroom, potato, squash, carrot, green bean, green pea, celery, leek, dill and parsley) to obtained a new functional and healthy product by enhancing the sum of all the bioactive power of the plant mixture (polyphenols, carotenoids, dietary fibers, vitamins and minerals) and its positive impact on the health. These authors concluded that addition of these vegetables mix was positively sensory acceptable and without affecting the overall quality, and therefore is suitable to obtain a healthy and functional processed cheese. Josipović et al. (2015) added fresh or dried spices (parsley, dill, pepper, garlic and rosemary) to improved properties and microbiological safety of cottage cheese. They reported that the biological value of novel cheese was improved by adding the plants rich in bioactive phenolic compounds, obtained higher total phenol content and free radical scavenging activity than the control sample. The differences in the phenolic content of the species and their contribution to the antioxidant activity of cheese were depended on several factors (pH, temperature, phenolic structure, molecular mass and amino acid composition) that would affect the interactions between phenolic molecules and proteins (Josipović et al., 2015). Other studies added plant extract, as an alternative option for food preservation, to enhance cheese quality (Pereira et al., 2018; Cosa et al. 2020). The passion fruit (*Passiflora cincinnata* Mast.) has been described as containing bioactive ingredient with antimicrobial activity and antioxidant potential (Leal, et al., 2018), and it has been used to control the growth of pathogenic bacteria often associated with dairy products. Cosa et al. (2020) added passion fruit in the production of coalho cheese, traditional Brazilian cheese, and concluded its potential use for controlling microbial populations in cheese due to the inhibitory potential of passion fruit.

2.3. Functional Ice creams

Ice cream is a widespread dairy product widely consumed by all age groups around the world mainly due to its taste characteristics, cooling effect as well as their nutritional benefits (Durmaz et al., 2020). These products are consumed at any time of the day with expected global sales of US $ 75 billion by 2024 (Bedford, 2022).

The concept of ice cream as a dessert encompasses a wide range of products that have in common the use of milk in their formulation. In general terms, the main ingredients that make up ice cream include 80% dairy products and less than 20% non-dairy fat ingredients (Loffredi et al., 2021). Non-dairy fat components such as sweeteners, emulsifiers, water, egg, stabilizers, minerals, colouring and flavouring agents and other ingredients are represented as dairy alternatives (Chuck-Hernandez et al., 2022). All these ingredients form a very complex, from physico-chemical point of view, food matrix which includes several physical phases. It consists of a mixture of air bubbles, fat globules, ice crystals, proteins, salts, sugars, and high molecular weight polysaccharides in a frozen-concentrated solution (Akbari et al., 2019, Arslaner and Salik, 2020).

On the other hand, it is important to note that ice creams have a high fat and sugar content that with an excessive consumption, can cause several health problems (Krystyjan et al. 2015). So, it would be interesting to functionalize this type of food, by reducing and/or replacing these ingredients or adding other ingredients that provide bioactive compounds that help to (*i*) adequately reduce these high levels of fat and sugars (*ii*) improve the physico-chemical and technological properties of ice creams. In general terms, the ice creams offered in markets shown lower contents in vitamins, natural antioxidants (carotenoids, phenolic acids, flavonoids) and pigments. In this sense, fruits,

vegetables and their coproducts might play a very important role for the enhancement of ice creams due to the their physico-chemical and organoleptic properties including sweet, taste, colour and flavour (Salehi, 2020) as well as for the bioactive compounds profile. Therefore, the enhancement of ice creams with the addition of fruits and vegetables which have a high content of healthy compounds was studied by several researchers (**Table 3**).

In this sense, Öztürk et al., (2018b) analyzed the phenolic content and the antioxidant activity of ice cream added, at 120 g/kg, with dark blue and white *Myrtus communis*. They found that after freezing process, the total phenol content value of reformulated samples increased by 5.00 and 8.50 mg gallic acid equivalents for dark blue and white *Myrtus communis* respectively. As regards to the antioxidant capacity, reformulated samples showed lower EC_{50} values, measure with DPPH methodology, than control sample due to the bioactive compounds added with *Myrtus communis*. Similarly, Mehditabar et al., (2020) investigated the effects of addition of pumpkin puree, at 10, 20 and 30%, on the bioactive compounds content, antioxidant and sensory properties of ice creams. They found that the addition of pumpkin puree increased total phenolic content, the dietary fibre, and antioxidant activity of ice creams and did it in a concentration-dependent manner. Sensorially, the pumpkin puree addition increased colour, intensity of fruit flavours and firmness of ice creams. In more recent study, David de Oliveira et al. (2021) carried out a study to analysed the bioactive compounds content (phenolic acids and flavonoids) and the antioxidant properties of symbiotic goat milk ice cream with the inclusion (200 g/kg) of umbu fruit (*Spondias tuberosa*). The authors reported that the samples added with umbu fruit had higher content of phenolic acids and flavonoids than control sample as well as a higher antioxidant activity measure with DPPH and ABTS methodologies. Similarly, Curti et al., (2021) and evaluated the effect of the addition of different concentrations (20 to 26%) of camu-camu pulp (*Myrciaria dubia*) on bioactive compound content and sensory acceptance of milk ice cream formulations. These authors informed that all formulations had high concentrations of antioxidant compounds and vitamin C and the formulation with the addition of camu-camu pulp lower than 24% had greater sensory acceptance. Nasr (2021) conducted a study to analyse the physical, chemical and sensory properties of ice cream fortified with sapota fruit pulp (*Manilkara zapota*) at 5, 10 and 15%. The obtained results indicated that there was an increase in the ice cream content of minerals such as calcium, phosphorous, potassium, iron, zinc and vitamins A and C by the increase the addition of sapota fruit pulp. In the work of Boyanova et al., (2022) ice cream added with 5%, 10% and 15% lingonberry (*Vaccinium vitis-idaea* L.) extract were evaluated as a source of anthocyanins and flavonoids with good antioxidant properties. They showed that there was a significant rise in antioxidant activity (measure with ABTS and FRAP assays) with increasing the concentration of the extract probably due to the bioactive components mainly anthocyanins.

In reference to the use of extracts obtained from coproducts, which are a very important source of bioactive compounds, Kurt and Atalar (2018) examined the elaboration of ice cream using quince seeds, at different concentrations (0.25, 0.50 and 0.75%), due to the high dietary fiber, polysaccharide and protein contents of this coproduct. The authors reported that the ice creams improved the protein and dietary fiber (mainly soluble dietary fiber) contents compared with control ice creams. Additionally, better melting properties and textural properties were obtained for reformulated ice creams. Similarly, Pelaes Vital et al., (2018) to obtain a product with functional characteristics added grape juice coproducts into ice cream at 2.5%, 5.0% and 10.0%. They reported that the ice creams containing grape juice coproduct had a higher concentration of phenolic compounds and antioxidant activity compared to the control samples and this occurred in a concentration-dependent manner. A similar trend has also been described by Böger et al., (2019) who

studied the antioxidant properties and bioactive composition of ice cream added, at 5, 10 and 15%, with jabuticaba (*Plinia cauliflora*) skin. The results obtained by these authors indicated that increasing concentration promoted an increase in the phenolic compound indices and the antioxidant potential. Utpott et al., (2020) apply a flour, as a fat substitute in ice creams, obtained from coproducts of red pitaya pulp processing. The addition of red pitahaya flour, which had a high content of fibre, into strawberry ice cream improved overrun and rheological behaviour of the sample with 73.5% fat reduction and resulted in a product with high overall acceptability. Another study was conducted by Mohammadian et al. (2020) to analysed the antidiabetic and antioxidant properties of ice cream elaborated with pistachio peel extract at different concentrations (10, 10 and 30%). These authors found that ice creams containing 20 and 30% of pistachio peel provided good antidiabetic properties due to the α-glucosidase inhibition achieved was 79 and 86%, respectively % while the antioxidant activity was improved between 6.2 and 9.4 times more than the control. Recently, Haghani et al., (2021) investigated the incorporation of cornelian cherry (*Cornus mas* L.) peel into ice cream at 3, 6, and 9% on bioactive compounds and antioxidant activity. These authors reported that the addition of cornelian cherry peel significantly enhanced vitamin C, total polyphenols, total anthocyanin content, and antioxidant activity of the ice cream.

2.4. Functional dairy desserts

Dairy desserts (excluding yogurts and ice cream) include a broad variety of products with many flavors, textures and presentations that contain large proportions of milk solids. They include creamy and gelled desserts, custards and puddings, sachet dessert mixes and cheesecakes (Saunders, 2022). In general, dairy desserts are complex mixtures and matrices including main components such as milk, sugar, starch, hydrocolloids, colorants, and flavors which, in turn, influence the nutritional, physical, and sensory characteristics, with direct effects on consumer acceptability. Thus, dairy desserts are well received globally and the market for such products has increased quickly with many ready-to-eat dairy desserts available for consumers (Singh et al., 2022). In this context, this kind of dairy products are an attractive option for incorporating potential functional ingredients obtained mainly from fruits and vegetables that add sensory and functional value (Kuriya et al., 2020). For example, Jridi et al., (2015) analysed a dairy dessert elaborated with date paste (*Phoenix dactylifera* L.) flours (16%) or date syrup as sugar and maltodextrin replacer. The authors informed that the reformulated dairy desserts had a higher total phenolic content and higher antioxidant capacity than control samples. In a similar and more recent research Djaoud et al., (2020) investigated the substitution of sugar by date syrup or date paste in a dairy dessert. They found that dairy dessert added with date syrup exhibited the highest total phenolic content, DPPH inhibition and reducing power than control sample. In an identical manner, Singh et al. (2022) carried out a study where the milk solids of dairy dessert were replaced by date (*P. dactylifera* L.) paste at 50% to 70%. These authors found that compared to control, inclusion of date pulp in dairy dessert formulations significantly increased the mineral content (mainly potassium and zinc), the total phenolics content, the ascorbic acid content and the antioxidant activity.

For their part, Ivanova et al., (2018) reported that dairy dessert added with encapsulated polyphenolic rich extract obtained from Cornelian cherry, chokeberry and blackberry showed higher antioxidant activity and higher bioactive compounds (mainly anthocyanins), than control samples. Lino et al. (2022) investigated the thermosonication effects on several quality parameters of a dairy dessert elaborated with jamun (*Syzygium cumin*) fruit. These authors found that the treated samples showed higher anti-

hypertensive (> 39%), antioxidant (>3 3%), and anti-diabetic (> 27%) activities, a higher concentration of phenolic compounds (> 22%), preservation of anthocyanins, and better digestibility due to the smaller fat droplet size. A recent study has shown that Burfi, an Indian traditional dairy dessert added with kinnow juice at (42%) showed higher content of minerals (magnesium) total phenolics acids and total flavonoids content as well as vitamin C compared to control. Additionally, the inclusion of kinnow juice significantly improved the antioxidant activity (Kaur et al., 2022).

3. Conclusions

The reformulation of dairy products such as yogurts, cheeses, ice creams and dairy desserts with extracts from fruits, vegetables and their co-products is a suitable strategy for the incorporation of bioactive compounds, including minerals, vitamins, dietary fiber, polyphenolic compounds and carotenoids in these products. The enrichment of dairy products with these natural sources of bioactive compounds enhances their nutritional and functional properties (due to the biological properties of these compounds) without negatively affecting, their technological and organoleptic properties. In addition, the valorization of coproducts from agro-industries contributes to the food chain sustainability through the circular economy.

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Table 1. Main bioactive compounds found in edible fruits and vegetables and their co-products

Fruits & vegetables	co-products	Bioactive compounds	Reference
Orange 	peel, pomace	dietary fiber, phenolic acids (chlorogenic, caffeic, ferulic and p-coumaric acid), and flavonoids (rutin, naringenin, hesperetin, and luteolin)	Arias et al., (2022); Aqilah et al., (2023)
Strawberry 	peel & pomace	dietary fiber, phenolic acids (gallic, vanillic, syringic, caffeic and p-coumaric acid), flavonoids (kaempferol, myricetin, catechin), anthocyanins (pelargonidin), and tannins (ellagic acid)	Arias et al., (2022); Aqilah et al., (2023)
Apple 	peel & pomace	dietary fiber, phenolic acids (chlorogenic acid), flavonoids (quercetin, rutin, kaempferol, myricetin, catechin, and epicatechin) and anthocyanins (petunidin)	Arias et al., (2022); Gulsunoglu et al., (2020)
Papaya 	peel, seeds & pomace	dietary fiber, carotenoids (cryptoxanthin, beta-carotene, lutein, and zeaxanthin), flavonoids (kaempferol, myricetin) and phenolic acids (vanillic, chlorogenic, ferulic, *p*-coumaric and caffeic acid)	Sharma et al., (2020); Singla et al., (2022)
Red grapes 	peel & pomace	dietary fiber, phenolic acids (gallic acid), flavonoids (rutin, kaempferol. catechin and epicatechin), anthocyanins (malvidin), and stilbenes (resveratrol)	Arias et al., (2022); Aqilah et al., (2023)
Blackberry 	peel & pomace	dietary fiber, phenolic acids (gallic, caffeic and *p*-coumaric acid), flavonoids (quercetin, luteolin), anthocyanins (pelargonidin), and tannins (ellagic acid)	Santos et al., (2019); Arias et al., (2022);
Date fruit 	peel & seeds	dietary fiber, carotenoids (lutein and beta-carotene) phenolic acids (caffeic, gallic, and syringic acid) and flavonoids (quercetin,	Masqood et al., (2020); Fernández-López et al., (2022)

		luteolin, rutin, and catechin) and tocopherols	
Blueberry 	peel & pomace	dietary fiber, phenolic acids (chlorogenic acid), flavonoids (quercetin, rutin, and myricetin), anthocyanins (delphinidin, cyanidin, malvidin, peonidin, and petunidin), stilbenes (resveratrol) and tannins (ellagic acid)	Arias et al., (2022); Aqilah et al., (2023)
Pumpkin 	peel & seeds	dietary fiber, carotenoids (lutein, zeaxanthin and β-carotene), tocopherols, flavonoids (rutin and kaempferol) and phenolic acids (gallic, protocatechuic, 4-hydroxybenzoic, vanillic, ferulic, *p*-coumaric and chlorogenic acid)	Kulczynski et al., (2019); Hussain et al., (2021)
Avocado 	peel & stone	dietary fiber, phenolic acids (trans-5-*O*-caffeoyl-D-quinic acid), flavonoids (catechin and epicatechin) and tannins (procyanidin)	Tremocoldi et al., (2018); Aqilah et al., (2023)
Beetroot 	peel, pomace & juice	dietary fiber, betalains (betacyanins and betaxanthins), carotenoids, flavonoids (betagarin, betavulgarin, cochiophilin A), saponins and phenolic acids (ferulic, caffeic, vanillic and syringic acid)	Chhikara et al., (2019); Fernández-López et al., (2023)
Artichokes 	bracts, exterior leaves & stalks	dietary fiber, phenolic acids (caffeic, chlorogenic and cynarin acid) and flavonoids (luteolin, apigenin, naringenin and narirutin)	Jiménez-Moreno et al., (2019)
Broccoli 	stalks	dietary fiber, glucosinolates (glucoraphanin, clucoiberin, glucobrasicin and neoglucobrassicin), isothiocyanates, phenolic acids (chlorogenic, caffeic and ferulic acid) and flavonoids (quercetin and kaempherol)	Le et al., (2020); Salas-Millán et al., (2022)
Asparagus 	leaves & roots	dietary fiber, saponins, flavonoids (rutin and quercetin) and anthocyanins (A1 and A2)	Guo et al., (2020); Aqilah et al., (2023)
Onion 	peel, external fleshy leaves, top & bottom part	dietary fiber, phenolic acids (vanillic, protocatechuic and ferulic acid) and flavonoids (quercetin), anthocyanins and tannins	Sagar et al., (2020); Kumar et al., (2022)

Potato 	peel	dietary fiber, flavonoids (catechin, quercetin and kaempferol), phenolic acids (caffeic, *p*-coumaric and ferulic acid), anthocyanins (malvidin, petunidin and pelargonidin) tanins and carotenoids	Joly et al., (2020); Makori et al., (2022)
Eggplant 	peel & calyx	dietary fiber and anthocyanins (cyanidin, peonidin, malvidin and delphinidin),	Doulabi et al., (2020); Karimi et al., (2021)
Pepper 	peel & seeds	dietary fiber, flavonoids (quercetin, luteolin and apigenin), phenolic acids (ferulic and sinapic acid), cpasaicinoids (capsaicin and dihydrocapsaicin) and carotenoids	Batiha et al., (2020) Fernández-López et al., (2020)
Tomato 	peel, seeds, & pomace	dietary fiber, phenolic acids (caffeic, chlorogenic, sinapic, *p*-coumaric and ferulic acid), flavonoids (naringenin, kaempferol, myricetin and quercetin), carotenoids (lycopene, α- and β-carotene) and glycoalcaloids (tomatine)	Viuda-Martos et al., (2014); Chaudhary et al., (2018)
Sweet potato 	peel & pomace	dietary fiber, anthocyanins, carotenoids (β-carotene) and phenolic acids (caffeic, chlorogenic and their isomers)	Gabilondo et al., (2022)
Carrot 	peel and pomace	dietary fiber, carotenoids (α- and β-carotene), phenolic acids (caffeic and anthocyanins (cyanidin, peonidin, pelargonidin and delphinidin)	Šeregelj et al., (2020)

Table 2. Incorporation of bioactive compounds from fruits, vegetables and their coproducts in yogurt.

Extract added (vegetable/fruit)	Addition target	Concentration	Effects	Reference
Peel of dragon fruit	Source of fibre and antioxidants	0, 25, 35, 45%	Higher fibre content, antioxidant activity and melting time	Analianasari & Apriyani (2017)
Wild herbs extract	Source of antioxidants	0, 0.25, 0.5, 0.75, 1%	Higher antioxidant activity and nutritional value	Dabija et al. (2018)
Chia mucilage	Fat replacer	0, 2.5, 5, 7.5%	Increased fibre content and improved nutritional value	Ribes et al. (2020)
Lentil flour	Source of proteins and fibre	0, 1, 2, 3, 4%	Increased mineral and protein content	Haq et al. (2019)
Rice bran	Effects during fermentation	0, 1, 2, 3%	Better stability, more compact and porous after addition of rice bran before fermentation	Wu et al. (2023)
Rosehip fortification	Antioxidant activity, phenolic compounds, and sensory properties	0, 5, 10, 15, 20%	Good source of phytonutrients (phenolics, natural sugar), the antioxidant activity improved with the addition of rosehip pulp to the yoghurt.	Sahingil & Hayaloglu (2022)
Soybean and quinoa	Sensory acceptance and nutrition value	Q:S - 10:0 - 8:2 - 6:4 - 4:6 - 2:8 - 0:10	Improved antioxidant properties	Huang et al. (2022)
Elaeagnus angustifolia L. flour	Effects on antioxidant properties	0, 1, 2%	Higher phenolic content and antioxidant activity	Öztürk et al. (2018a)
Mucilage-free flaxseed	Source of fibre, protein, and omega-3 fatty acids	0, 3%	Improved viscosity and decreasing syneresis	Basiri et al. (2022)
Chia seeds and strawberries	Source of fibre	Chia seeds: 0, 6, 14, 6, 14, 10% Strawberries: 8, 8, 12, 12, 10%	Increased the levels of protein, lipids, dietary fiber, and polyunsaturated fatty acids, especially the ω-3, and mineral content.	Kowaleski et al. (2020)

Chia seeds	Improved nutritional value	0, 2, 3, 4%	Increased protein content and improved fatty acid profile	Eker & Karakaya (2020)
Lentil flour	Functional ingredient	0, 4%	Low syneresis	Benmeziane et al. (2021)
Carrot extract	Functional product due to β-carotene	0, 2.5, 5%	High β-carotene content and improved antioxidant activity	Seregelj et al. (2021)
Pilosocereus gounellei flour	Potential effect as an additive	0, 1, 2%	Higher mineral, total phenolic compounds, and flavonoid contents and greater antioxidant activity	Dantas et al. (2022)
Rice and oats extract	Effects in the fermentative process	50% replacement	Source of ω-6 and ω-9	Campos et al. (2018)
Chia seed extract	Increased phenolic compounds, dietary fibre and ω-3 fatty acids	0, 0.05, 0.1%	Shortened fermentation time and increased LAB counts. In addition, it improved physicochemical properties and antioxidant effects	Kwon et al. (2019)
Banana and banana peel fibre	Source of fibre	0, 0.2, 0.5, 1%	Higher fibre content	Safdari et al. (2021)

Table 3. Incorporation of bioactive compounds from fruits, vegetables and their coproducts in cheese.

Dairy product	Extract added (vegetable/fruit)	Addition target	Concentration	Effects	Reference
Probiotic Cheese	Broccoli paste (*Brassica oleracea*)	Source of bioactive compounds	5, 10 and 15%	Decreased fat, protein and ash Increased contents phenols, antioxidant activity, minerals and vitamins,	Abd El-Montaleb et al., (2022)
Soft cheese	Spinach (*Spinacia oleracea*) powder	Source of functional compounds	0.50, 1.00, 1.50, and 2%	Improved content of fiber, minerals, total phenolic content, and antioxidant activity was Increased the total solid, protein and acidity Lower values in L*, a*, and b* coordinates Lower sensory acceptability	El-Sayed (2020)
Processed cheese	Spirulina Powder (*Spirulina máxima)*	Source of nutrients, antioxidants and color	1, 2 and 3%	Higher green (a-value) and lower whiter (L-value) Higher free radical scavenging activity Increased the protein, carbohydrates and fiber contents (ratio 3%)	Mohamed et al., (2020)
Functional processed cheese	β-carotene extract (CE) and β-carotene nano-encapsulation (CND) from carrot coproduct	Source of β-carotene	0.3% CE, 0.2% of 10% CNE 0.3 % of 15% β-CND	Carotenoid nanoparticles were more stable than the free extract. Higher nutritional value, sensory acceptability and economical advantages in Cheese with carotenoid nanoparticles	Basuony et al., (2022)
Queso Blanco Cheese	Tomato Extracts (powdered microcapsules)	Source of health and bioactive compounds	0.5-2.0%	Higher lycopene concentrations Lower lactic acid bacterial count Improved sensory evaluation scores Increased yellowness and tomato taste.	Jeong et al., (2017)
Processed Cheese	Tomato juice	High nutrition and healthy effect.	10, 20 and 30%	Higher Potassium Presence of Ferrous and lycopene Higher Residual Scavenging Activate (RSA%) and phenolic compounds	Mehanna et al., (2017)

Processed Cheese	Vegetable mix (mushrooms, dill, leeks, parsley, celery, green peas, squash, potatoes, and carrots)	Fortification of chees	2.5, 5, 7.5, and 10%	Increased in amino acid content, poly-unsaturated fatty acids, total phenolic compounds and radical scavenging activity Increased in Vitamin A, Vitamin C, vitamin B complex (B1, 2, 3, 6) vitamins. Increased in minerals (K, Mg, Se, and Fe)	El-Loly et al., (2022)
Processed Cheese	Sweet Red Pepper Paste	Source of phenol compounds and ascorbic acid	10% and 20%	Increased the total solids, fiber, protein, ash, fat, pH, potassium, total phenolic contents, and Radical Scavenging Activity. Increased chewiness and springiness	Atwaa et al., (2020)
Cream Cheese	Mushroom (*Agrocybe aegerita)*	Improved nutritional properties	3%	Enhanced the overall nutritional value, taste and smell of cream cheese Good acceptance by panellists	Petrovic et al., (2015)
Processed Cheese	Fresh and dried mushroom	Improved nutritional and functional value	0, 5, 10 and 15% fresh mushroom 0, 1, 1.5 and 2% dried mushroom	Decreased viable counts and spore former bacteria Increased moisture, ash, minerals and protein Increased in lipolytic and proteolytic bacteria Increased texture score	Khider et al., (2017)
Processed Cheese	Dried mushroom, potato, squash, carrot, green pea, celery, leek, dill and parsley	Source of nutritional and functional components	2.5, 5.0, 7.5 and 10%.	Nutritional enhancement (higher in dry matter, protein, fiber and carbohydrates). Increased the hardness values during storage. Good acceptance during storage.	Farahat et al., (2021)
spreadable Cheese	Flours from grape pomace, tomato peel, broccoli, corn bran, and artichokes	Sources of fibres and antioxidant compounds	5%	Increased the total phenolic content and flavonoids.	Lucera et al., (2018)
Coalho cheese	Passion fruit (*Passiflora cincinnata*)	Biopreservative	3%	lower counts of mesophilic aerobes, reduction of *Staph. aureus* counts	Costa et al., (2020)

Table 4. Incorporation of bioactive compounds from fruits, vegetables and their coproducts in ice creams.

Extract (vegetable/fruit) added	Addition target	Concentration	Effects	Ref.
Apple, oat, and wheat fibers	Source of fiber	2 and 4%	Enhance nutritional and physiological aspects. Reduction of melting point	Soukoulis et al., (2009)
Pomegranate peel extract or grape seed extract	Source of antioxidants	0.4%	Increased total phenolic content of ice creams. The antioxidant activity of supplemented ice creams were higher than that of control group	Sagdic et al., (2012)
Kiwifruit	Source of antioxidants	49%	Increased the polyphenols and vitamin C contents in addition to the natural color flavor of the kiwifruit added.	Sun-Waterhouse et al., (2013)
Peel, bagasse, and orange seed coproducts	Source of fiber and fat replacer	1 and 1.5%	Reduction of fat content (50 %) and the overrun ratio. Increased the fiber content and the hardness, gumminess, and springiness values	Crizel et al. (2014)
Persimmon puree	Source of bioactive compounds	8, 16, 24, 32, and 40%	Improved the quality of samples in terms of sensory and bioactive properties. Higher content in minerals than control	Karaman et al., (2014)
Banana peel and pulp flour	Source of fiber and minerals	1 and 2%	Increased fiber content. Improved the mineral profile	Yangılar (2015)
Sugarcane juice	Antioxidant	20, 40, and 60%	Improved the antioxidant activity. Inhibition of oxidation of unsaturated fatty acids during storage for 180 days	Ullah et al., (2015)
kumquat (*Fortunella margarita*)	Source of bioactive compounds	5,10,15%	Increased in acidity, vitamin C content, b^* value, and overrun value compared with the control ice cream	Çakmakçı et al., (2016)
Cornelian Cherry (*Cornus mas* L.) Paste	Source of antioxidants	5, 10, and 15%	Increased vitamins and flavonoid content. Improved the antioxidant activity.	Topdaş et al., (2017)

Apple, orange, oat, wheat, and bamboo fibers	Source of fiber	2%	Reduction in fat content and melting values. Increased the survival of probiotic bacteria	Akalın et al. (2017)
Pindo palm fruit (*Butia odorata*)	Source of antioxidants	30, 40, and 50%	Carotenoids and flavonoids increased during frozen storage. Sensory analyses showed that butiá ice cream had high acceptability	dos Santos Cruxen et al (2017)
Golden Berry Juice	Source of antioxidants	3, 6 and 10%	Increased antioxidant activity. Improved bioactive compounds profile. High content of Vitamin C and minerals mainly Fe, K, and Zn	Naeem et al., (2019)
Sloe Berry (*Prunus spinosa* L.)	Source of bioactive compounds	5, 10 and 15%	Improved the potassium and Manganese contents. Antioxidant activity of ice cream samples were increased.	Ürkek, et al (2019)
Colocasia esculenta Stem	Antioxidant	6%	Improved the antioxidant activity. High content in Vitamins C and E and minerals	Asaduddin et al., (2021)
Nabq fruit pulp (*Ziziphus spina-christi* L.)	Source of bioactive compounds	25, 50, 75 and 100%	For all concentrations, the ice cream had a higher concentration of phenolic compounds and higher antioxidant activity compared to the control samples at zero time and after 40 days	Tawfek et al., (2022)

7.5. PUBLICACIÓN 5

Fortification of goat milk yogurts with date palm (*Phoenix dactylifera* L.) coproducts: Impact on their quality during cold storage

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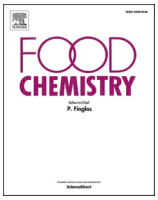
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Fortification of goat milk yogurts with date palm (*Phoenix dactylifera* L.) coproducts: Impact on their quality during cold storage

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ABSTRACT

The aim of this study was to investigate the impact of different concentrations (3% and 6%) of two ingredients (paste and flour) obtained from the valorization of date fruit coproducts on the nutritional (proximate composition and mineral profile), technological (coagulation curve, pH, acidity, sugar and organic acid content and syneresis), physicochemical (color, water activity and texture), microbiological and sensory properties of goat's yogurt during 21 days of refrigerated storage. Both ingredients enhanced the growth and stability of the yogurt starter culture, thereby improving the probiotic potential of date-added yogurts. Physicochemically, the addition of date flour (at both concentrations) induces stronger modifications (texture, color and syneresis) in yogurts than the date paste. During storage, date paste reduced the syneresis and hence maintained yogurts' physical quality. Consumers preferred the yogurts with date paste (3% and 6%) rather than with date flour, because its addition led to a more brownish color and granular texture.

1. Introduction

Milk has been part of the human diet for decades due to its outstanding nutritional properties and health benefits. In addition, the wide versatility of milk along with its high acceptance among the consumers has caused the dairy industry to become one of the leading sectors in the development of functional foods (Verruck et al., 2019). Yogurt is a well-known and commonly consumed dairy product and is characterized by a high nutritional value, even higher than other non-fermented dairy products, due to its high vitamin B, conjugated linoleic acid and bioactive peptides contents, as well as enhanced protein digestibility and calcium bioavailability (Fardet, Dupont, Rioux, & Turgeon, 2019). These properties have been mainly assessed in yogurts made with cow milk, but they can be improved using goat milk mainly due to its greater digestibility caused by the high proportion of small fat globules (1.5 μm) and lower allergenic properties compared to cow milk (Milani & Wendorff, 2011). When compared to cow milk, goat milk contains higher levels of mineral elements like zinc, iron and magnesium, short-chain fatty acids, and antibacterial compounds (Slačanac et al., 2010). In addition, yogurt contains beneficial lactic acid bacteria with probiotic potential that can provide health benefits. From a physico-chemical perspective, yogurt is an acid milk gel in which casein micelles aggregate and form a network entrapping serum inside. This network is strengthened by the formation of complexes between denatured whey proteins due to heat treatment, k-caseins found on the surface of casein micelles, and fat globules. This process occurs as milk is acidified through fermentation with lactic acid bacteria, thereby reducing the negative charge of casein micelles. The milk acidification is neutralized at the isoelectric point of 4.6 with the consequent reduction in the electrostatic repulsions (Durmus, Capanoglu, & Kilic-Akyilmaz, 2021). The key market constraints for goat milk products stem from the distinctive aroma and flavor of goat milk, which is stronger than that of cow milk and is attributed to its content of capric, caprylic, and caproic fatty acids (Slačanac et al., 2010). Additionally, goat milk products often exhibit a weaker curd formation and lower viscosity due to reduced levels of α-s1 casein (Costa et al., 2017; Wang et al., 2023). In this way, the addition of some sweeteners, texturizers, colorants and flavoring compounds have been commonly used in order to improve

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both the rheological properties and the "goaty" flavor in goat's milk yogurts. Nowadays, in line with the emerging trend for more natural foods, these effects could be addressed using fruit and fruit extracts.

Fruits and fruit extracts have been used for the fortification of yogurts improving not only their chemical composition and health benefits but also acting as a natural stabilizer, coloring and texturizing agent to improve their microstructure, color and texture (Chen et al., 2023; Wang, Kristo, & Lapointe, 2019; Wu, Deng, Luo, Liu, & Hu, 2023). Strawberry-tree fruit, cherry, mango, figs, pomegranate and, cactus pears (opuntias) are examples of fruits that have been used for the development of functional yogurts. These fruits have been added mainly as extracts either directly or previously encapsulated (Ramli, Ali, Hamzah, & Yatim, 2021; Rashwan et al., 2022, Rashwan et al., 2024; Šeregelj et al., 2021) as a way to increase the stability and improve the homogenous distribution of compounds. In most cases solvents such as ethanol, hydrochloric acid or n-hexane, in combination with ultrasounds or microwaves or other technologies have been used for extract's preparation. Furthermore, they can be incorporated before or after fermentation, which could affect the development of the bacterial starter cultures and consequently the fermentation process.

The production and marketing of fresh Confitera dates in Spain has increased considerably as a result of promotional campaigns both for their growth characteristics (an autochthonous cultivar, well adapted to the specific edaphoclimatic conditions found in the European oasis, eco-efficient in their growth and with a sustainable production) and for their nutritional and healthy properties (Fernández-López, Viuda-Martos, Sayas-Barberá, Navarro-Rodríguez de Vera, & Pérez-álvarez, 2022). Besides, dates are a product linked to the territory contributing to the development of the local economy and a good example of circular economy. Their commercialization can generate an important amount of coproducts (about 30% of dates are discarded or wasted due to low-grade classification (size, color, insects o natural damages, etc.) which can be valorized applying simple and environmentally friendly technologies obtaining several types of intermediate foods products with high added-value (date pastes, date waters and date flours) and different appearance, physicochemical properties and composition, giving them a great versatility for their incorporation in different food matrices (Muñoz-Bas et al., 2023). Therefore, some of these high-added value products obtained from dates valorization could be directly added to yogurts, without any additional treatment, obtaining date-enriched yogurts. The development of goat yogurts containing date coproducts should be approached from a global perspective, considering not only the nutritional composition, but also their technological and sensory properties. The yogurt matrix was selected as it is consumed worldwide and can be easily incorporated in daily meals without changing dietary patterns. Moreover, these date coproducts could improve goat yogurt texture and stability avoiding added sugars.

The aim of this work was to assess the impact of different concentrations (3% and 6%) of intermediate food ingredients (date paste and date flour) obtained from the valorization of date coproducts (Confitera cv.) on the nutritional, technological, physicochemical, microbiological and sensory properties of goat's yogurt during cold storage.

2. Material and methods

2.1. Materials

Goat semi-skimmed milk was purchased from a local supermarket and stored at 4 °C until use; the commercial starter YO-MIX® 495 LYO 100 DCU was obtained from Danisco (Copenhage, Denmark). Date paste and flour were obtained from Confitera cv. dates at tamar stage following the procedure described by Muñoz-Bas et al. (2023). The full characterization of both ingredients (chemical composition, physicochemical properties and microbial quality) has been previously assessed by these authors (date paste: 48% moisture, 31% sugars, 19% TDF, 1.1% proteins, 0.5% ash and 0.4% fats; date flour: 66% TDF, 19% sugars, 7% proteins, 6% moisture, 1.7% ash and 1.1% fats).

2.2. Yogurt making

Goat milk (8 L) was divided into 5 parts (1600 mL), then either date paste or date flour were added at 3% and 6% (*w*/w) in four of the portions, being the fifth the control sample. Date paste or flour were incorporated into the milk at the established concentrations homogenizing the mixture until it reached 42 ± 2 °C. Once the temperature was reached, the yogurt starter was incorporated at the concentration indicated by the manufacturer (10–20 DCU/ 100 L) and homogenized for 2 min. The samples were distributed in sterile 100 mL containers (16 per each batch) and placed in an incubator at 42 ± 2 °C until the pH reached between 4.65 and 4.60. Finally, the yogurts were stored at 4–6 °C during 21 days for the subsequent analyses. Yogurt manufacturing was performed in triplicate in three independent sessions to assure reproducibility of results.

2.3. Yogurt analysis

2.3.1. Proximate composition of yogurts

The composition of the yogurts was analyzed in triplicate one day after their production (time 0 d). Fat, protein, and total solids content was measured with a MilkoScan FT120 (FOSS, Denmark) calibrated for cream, while moisture (AOAC 925.45) and ash (AOAC 923.03) were determined following AOAC methods (AOAC, 2006).

2.3.2. Mineral composition of yogurts

The effect of the addition of date (paste and flour) on the yogurt's mineral composition was analyzed using inductively coupled plasma–mass spectrometry (ICP-MS) Shimadzu MS-2030 (Shimadzu, Kioto, Japan) using the same conditions as described by Muñoz-Bas et al. (2023). Briefly, samples were lyophilized (Freeze dryer Alpha 2–4, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and the mineral content was quantified after digestion with nitric acid (67%) and hydrogen peroxide (33%) using a microwave system. The results were the average of 3 reads and expressed as mg/100 g dry weight of yogurt samples.

2.3.3. Organic acids and sugar composition of yogurts

The extraction and quantification of sugars and organic acids of the yogurts were performed using the same method as described by Muñoz-Bas et al. (2023). Briefly, 2 g of samples were mixed with 50 mL of ultrapure water, homogenized at 20,000 rpm for 2 min (Ultra-Turrax T25 BASIC, IKA-Werke GmbH & Co. KG, Staufen, Germany), heated at 80 °C for 1 h and centrifugated (6500 ×g for 10 min at 4 °C) recovering the supernatant and filtering through a 0.45-μm filter. Organic acids and sugars were analyzed by injecting 20 μL of sample in an HPLC (Hewlett-Packard 1100 series model, Woldbronn, Germany) provided with a Supelco column (Supelcogel TM C—610H column 300 mm × 78 mm). Ortho-phosphoric acid in water (0.1% *v*/v) was used as elution buffer at a flow rate of 0.5 mL/min. Organic acid were determined by measuring the absorbance at 210 nm with a diode-array detector (DAD G-1315 A), while sugar determination was carried out by using a refractive index detector (RID G1362A). Peaks were then identified by comparing with the standards (organic acids, monosaccharides, and oligosaccharides from Supelco, Sigma-Aldrich, St. Louis, MO, USA) retention time and quantified by the regression formula obtained with the standards. These determinations were assessed at time 0 and at the end of refrigerated storage (21 days).

2.3.4. Physicochemical analysis of yogurts

The physicochemical properties of yogurts were assessed at time 0 and after 7, 14 and 21 days of cold storage (4–6 °C).

2.3.4.1. pH and titratable acidity. The pH of the yogurts was measured with a Crison GLP 21 pH meter (Crison, Barcelona, Spain) and the titratable acidity was determined by titration with NaOH 0.11 N using phenolphthalein as an indicator, expressing the results as ^{o}Dornic (^{o}D).

2.3.4.2. Syneresis. Syneresis of the samples was determined according to the method described by Jrad, Oussaief, El-Hatmi, and Bouaziz (2022) using 50 mL of yogurt. The percentage of syneresis was calculated with the following equation:

$$\%\text{Syneresis} = (V_1/V_0)\ \text{x}\ 100$$

where V_0 is the initial volume (mL) of yogurt and V_1 is the volume (mL) of the whey recovered after drainage.

2.3.4.3. Color properties. CIELAB color coordinates [lightness (L+), red/green coordinate (a*), and yello-green coordinate (b*)] were measured with a Minolta CM-700d spectrophotocolorimeter (Konica Minolta, Osaka, Japan) selecting D65 illuminant and 10^{o} observer angle. From these coordinates, the psychophysics magnitudes [chroma (C*) and hue (h*)], and the whiteness index [$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$] (Akgün et al., 2020) were calculated. All measurements were performed in triplicate.

2.3.5. Mechanical properties of yogurt (texture)

Textural properties of the yogurts incorporated with date was measured during 21 days of refrigerated storage following the method described by Silva and O'Mahony (2018) with slight modifications. The back extrusion test was carried using a Texture Analyser TA-XT2i (Stable Micro Systems Ltd., Godalming, Surrey, UK), equipped with a 5 kg load cell. Yogurts were manufacture in 100 mL sterile polypropylene containers (diameter = 55 mm; height = 70 mm) and stored at 4 °C until analysis at 7-day intervals. A cylindrical prove (Ø = 10 mm), operating at a fixed test speed of 1.0 mm/ reaching up to 30 mm depth was used and the measurements were performed at 25 °C. The textural parameters were measured in triplicate and the results were expressed as: the maximum positive force in compression (firmness in N), the positive area of the curve (texture graph, force vs time) that indicates the internal strength of bonds within the product (consistency in N*s), the maximum negative force of the curve that indicates the force required to remove the probe from the sample (cohesiveness in N) and the negative area of the curve (viscosity index in N*s).

2.3.6. Microbiological quality

The effect of the date coproducts incorporation on the yogurt starter culture was evaluated during 21 days of refrigerated storage at 7-day intervals by measuring the viable cell numbers of *Lactobacillus* spp. and *Streptococcus* spp. To estimate the population of *Lactobacillus* spp. and *Streptococcus* spp., 10 g of sample were homogenized with 90 mL of sterile peptone water 0.1% (w/vol) in a masticator for 60 s. Then, decimal dilutions were prepared using the same medium and, 0,1 mL was manually seeded, in duplicate, on MRS agar for *Lactobacillus* spp. and on M17 agar for *Streptococcus* spp. After that, petri dishes were incubated at 37 °C for 48 h in the case *Streptococcus* spp., and at 37 °C for 48 h in an anaerobic chamber with an Anaerocult A (Merck, Darmstadt, Germany) for *Lactobacillus* spp. To assess the adequate hygienic conditions during the yogurt making, counts of molds and yeasts, and enterobacteria were also determined using the same dilutions previously described. One mL of these dilutions was seeded, in duplicate, in Petrifilm plates (3 M, Madrid, Spain) for molds and yeasts and for enterobacteria. Pertrifilm dishes were incubated at 37 °C for 24 h for enterobacteria, and at 25 °C for 120 h for molds and yeasts. Plates (30–300 colony-forming units (CFU)) were manually counted expressing the results as log CFU/g of yogurt.

2.4. Sensory analysis

To evaluate the sensory acceptability of the yogurts, twenty-six consumers (65% female and 35% male, aged between 18 and > 65 years) were recruited at the Orihuela Polytechnic School of the Miguel Hernández University (UMH). Before starting the analyses, each subject was informed about the specific characteristics of the product to be tasted and about what the analysis would consist of, and a written informed consent was signed by the participants. This project was approved by the Responsible Research Office of the Miguel Hernández University (OIR- Reg. 211,128,200,759, Ref. PRL.DTA.JPA.05.21, UMH, Elche, Alicante, Spain). All sensory work was carried out on freshly prepared yogurts (day 0) at the standardized sensory laboratory of the UMH which fulfills the requirements according to the international standards (ASTM, 1986). During evaluation the subjects were seated in private booths under TL 5 fluorescent light (Philips-Ibérica, Madrid, Spain), with an intensity of approximately 350 lx. Yogurt samples (10 g) were coded with a random 3-digit number and served blinded in a transparent plastic cup in a completely randomized order. Consumers were asked to rate their preference for odor, taste, color, sweetness, acidity, firmness, granularity and general acceptability. Preference was expressed on a 9-point hedonic scale ranging from "dislike extremely" (1) to "like extremely" (9) (Lawless & Heymann, 2010).

2.5. Statistical analysis

Statistical analysis of data was performed using SPSS (IBM SPSS Statistics version 26). Data were checked for normality and constant variance before applying ANOVA. One-way ANOVA was calculated (using a confidence level of 95%) to determine any significant difference between the control yogurt and the ones containing date paste (3 and 6%) and flour (3 and 6%). Two-way ANOVA was applied (using a confidence level of 95%) to evaluate the yogurts stability along time (using treatment and storage time as fixed effects). When there was a significant difference ($p < 0.05$), Tukey test was carried out to determine the differences among the different yogurt formulations and storage times.

3. Results and discussions

3.1. Coagulation curve during yogurt making

The addition of date flour (at both concentrations) significantly decreased ($p < 0.05$) the yogurt pH at the moment of its addition, which is related to the lower pH of the date flour (6.22; Muñoz-Bas et al., 2023), in contrast with the pH of date paste (7.16; Muñoz-Bas et al., 2023), whose addition resulted in the same pH ($p > 0.05$) than the control yogurt. The pH decrease was faster - and therefore more pronounced – in yogurts incorporated with date coproducts (both flour and paste and at both concentrations) than in control yogurt. The required final pH was reached at 5.30 h in yogurts added with date flour (3 and 6%) and 3% date paste, at 6 h in yogurts added with 6% date paste and at 7 h in control yogurt. During normal yogurt fermentation, lactic acid bacteria (LAB) produce lactic acid by fermenting milk carbohydrates causing an acidification or pH drop that leads to destabilization and gelation of milk proteins - mainly caseins - giving the texture characteristic of yogurt (Kanauchi, 2019). The incorporation of date products in yogurts could have favored an early formation of the protein network thus explaining the rapid acidification in these batches. Furthermore, providing extra carbon sources to the starter culture could accelerate the acidification process, thereby reducing fermentation time.

3.2. Proximate composition of date yogurts

Table 1 shows the results for the proximate composition of yogurts with the added date coproducts. No differences in protein and ash content between samples were detected ($p > 0.05$). However, the

Table 1
Nutritional composition of goat yogurts fortified with date coproducts (mean ± sd).

	Yogurt				
	Control	3% Date flour	6% Date flour	3% Date paste	6% Date paste
Moisture (%)	90.98 ± 0.35^{a}	89.61 ± 0.20^{b}	87.91 ± 0.22^{c}	90.72 ± 0.51^{a}	89.95 ± 0.56^{ab}
Ash (%)	0.89 ± 0.01^{a}	0.95 ± 0.02^{a}	0.98 ± 0.05^{a}	0.90 ± 0.03^{a}	0.93 ± 0.01^{a}
Protein (%)	2.57 ± 0.01^{a}	2.69 ± 0.10^{a}	2.77 ± 0.01^{a}	2.51 ± 0.04^{a}	2.56 ± 0.14^{a}
Fat (%)	1.92 ± 0.03^{b}	2.04 ± 0.03^{ab}	2.07 ± 0.01^{ab}	2.16 ± 0.08^{a}	2.19 ± 0.01^{a}
Total solids (%)	2.56 ± 0.06^{b}	3.77 ± 0.07^{ab}	4.11 ± 0.66^{a}	3.03 ± 0.07^{ab}	3.74 ± 0.23^{ab}

a-c Different letters in the same row indicate significant differences ($p < 0.05$) due to date addition.

addition of date flour decreased ($p < 0.05$) moisture content in a concentration-dependent manner (6% date flour yogurts showed lower moisture than 3% date flour yogurts) but the addition of date paste did not modify moisture content compared to control yogurts.

On the contrary, date paste yogurts showed higher fat content than control, without differences ($p > 0.05$) among their concentrations (3 and 6%). The differences found in the proximate composition between samples are mainly due to the composition of the date products added and, even though these were statistically significant, it is not important from a practical point of view since the moisture content ranged from 87.91 to 90.98% (*w*/w) and fat between 1.92 and 2.19%.

3.3. Mineral composition of yogurts

The contents of macroelements (Ca, K, P, Mg and Na) and microelements (Fe, Mg, Cu and Zn) in the analyzed yogurts are presented in Table 2. The addition of the date coproducts (paste and flour) before the fermentation process probably contributed to the variation of mineral contents between products. In general, it could be said that the fortification of yogurts with date coproducts significantly improved ($p < 0.05$) the concentration of K, Mn, Cu and Zn, decreased the concentration of Ca, Na and P, and had no effect on Mg and Fe concentrations. Date coproducts are rich in K, Mn, Cu and Zn (Muñoz-Bas et al., 2023) explaining their higher concentrations in date yogurts than in control. The content of magnesium in all yogurts ranged from 101.41 and 116.21 mg/100 g, and the content of iron between 0.99 and 1.12 mg/100 g, which is in agreement with the values reported for goat yogurts (Jrad et al., 2022).

3.4. Sugars and organic acids of yogurts

Table 3 shows the sugar and organic acids concentration of the date-added yogurts at time 0 and at the end of the refrigerated storage (21 days). Both compounds have been highlighted as relevant indicators of bacterial metabolic activity in yogurts, contributing to their taste and flavor along with other volatile and semi-volatile compounds (Adhikari, Grün, Mustapha, & Fernando, 2002). The main sugar in all the yogurts

Table 3
Sugar and organic acid concentrations (mg/g) of goat yogurts fortified with date coproducts during 21 days of refrigerated storage (day 0 and day 21).

	Organic acids		Sugars		
	Citric acid	Lactic acid	Lactose	Galactose	Glucose
			Storage time: Day 0		
Control	4.99 ± 0.02^{bA}	18.74 ± 0.10^{cB}	36.17 ± 0.20^{cA}	20.45 ± 0.28^{bA}	Nd
3% Date paste	4.95 ± 0.16^{bA}	17.71 ± 0.13^{dB}	40.16 ± 2.08^{bA}	18.42 ± 0.93^{bA}	9.83 ± 0.52^{dA}
6% Date paste	4.19 ± 0.19^{cA}	17.65 ± 0.11^{dB}	45.08 ± 3.52^{aA}	25.73 ± 2.31^{aA}	15.21 ± 0.18^{bA}
3% Date flour	5.14 ± 0.18^{aA}	19.50 ± 0.14^{bB}	45.17 ± 2.39^{aA}	23.65 ± 1.13^{aA}	12.79 ± 0.15^{cA}
6% Date flour	4.74 ± 0.10^{bA}	21.87 ± 0.21^{aB}	39.91 ± 1.38^{bA}	24.82 ± 0.89^{aA}	17.83 ± 0.5^{aA}
			Storage time: Day 21		
Control	5.07 ± 0.11^{aA}	20.41 ± 0.23^{cA}	31.44 ± 0.57^{cB}	20.89 ± 0.75^{cA}	Nd
3% Date paste	4.91 ± 0.13^{aA}	20.82 ± 0.60^{cA}	27.25 ± 1.67^{dB}	19.76 ± 0.32^{cA}	9.33 ± 0.19^{dA}
6% Date paste	4.09 ± 0.15^{cA}	21.42 ± 0.18^{bA}	25.23 ± 0.9^{eB}	24.57 ± 0.20^{bA}	14.55 ± 0.42^{cB}
3% Date flour	5.15 ± 0.06^{aA}	21.73 ± 0.62^{bA}	33.37 ± 0.64^{bB}	24.66 ± 0.10^{bA}	12.03 ± 0.47^{bB}
6% Date flour	4.78 ± 0.02^{abA}	25.64 ± 0.08^{aA}	36.63 ± 0.78^{aB}	25.29 ± 0.15^{aA}	17.52 ± 0.75^{aA}

a-d For the same compound and storage time, different lowercase letters in the same column indicate significant differences ($p < 0.05$) due to date addition.
A-B For the same compound and yogurt sample, different uppercase letters in the same column indicate significant differences ($p < 0.05$) due to storage time.
Nd: Not detected.

was lactose, followed by galactose ($p < 0.05$). Glucose was only detected in date-added yogurts, which means that its presence in goat milk yogurts is attributed to the addition of date coproducts (paste and flour). Paste-added yogurts showed lower glucose concentration than flour-added yogurts, and in both cases, as expected, an increased concentration of date coproducts in yogurt was associated with a higher concentration of glucose ($p < 0.05$). Glucose has been reported as the main sugar in Confitera date fruits (Muñoz-Bas et al., 2023). Lactose decreased with storage time ($p < 0.05$) but at different intensity levels. Yogurts with date paste added (3 and 6%) were associated with higher lactose degradation (32 and 44%, respectively) as indicated by the highest bacterial viable counts (see point 3.5.3, Fig. 1). No significant differences in galactose concentration during storage time were found in any of the analyzed yogurts ($p > 0.05$) which agrees with the results reported by other authors (Bertolino et al., 2015; Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011). The fact that the yogurt starter culture (*L. bulgaricus* and *S. thermophilus*) uses glucose but not galactose (O'Brien, 1999) would explain these results. The concentration of lactose and galactose in all yogurts ranged between 25.23 and 45.17 mg/g and 18.42–25.73 mg/g, which is in line with normal values for plain goat milk yogurts (Wang et al., 2023).

As expected, lactic acid was the major organic acid, showing a significant increase during storage in all yogurts ($p < 0.05$). Regardless of yogurt formulation, the higher increase was observed in yogurts added with 6% date paste (21.3% increase) and 3% date paste (17.5%

Table 2
Mineral composition (mg/100 g) of goat yogurts fortified with date coproducts (mean ± sd).[a-d]

Yogurt	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Control	876 ± 25^{a}	0.11 ± 0.01^{c}	1.12 ± 0.03^{a}	823 ± 30^{b}	116 ± 7.3^{a}	0.11 ± 0.01^{c}	412 ± 12^{a}	2621 ± 50^{a}	2.35 ± 0.06^{c}
3% Date paste	844 ± 16^{b}	0.23 ± 0.02^{b}	1.01 ± 0.03^{a}	999 ± 62^{a}	107 ± 5.3^{a}	0.22 ± 0.02^{b}	387 ± 2.4^{b}	2197 ± 66^{b}	3.39 ± 0.14^{b}
6% Date paste	755 ± 12^{c}	0.33 ± 0.01^{a}	1.03 ± 0.02^{a}	962 ± 25^{a}	113 ± 2.6^{a}	0.30 ± 0.03^{a}	367 ± 6.1^{c}	2013 ± 50^{c}	3.19 ± 0.47^{b}
3% Date flour	775 ± 13^{c}	0.17 ± 0.04^{b}	0.99 ± 0.09^{a}	958 ± 15^{a}	112 ± 4.0^{a}	0.24 ± 0.02^{b}	390 ± 6.5^{b}	2229 ± 39^{b}	4.05 ± 0.35^{a}
6% Date flour	666 ± 11^{d}	0.36 ± 0.01^{a}	1.04 ± 0.09^{a}	987 ± 46^{a}	101 ± 8.1^{a}	0.36 ± 0.02^{a}	340 ± 1.9^{d}	1811 ± 41^{d}	3.89 ± 0.09^{a}

[a-d] Different letters in the same column indicate significant differences ($p < 0.05$) due to date addition.

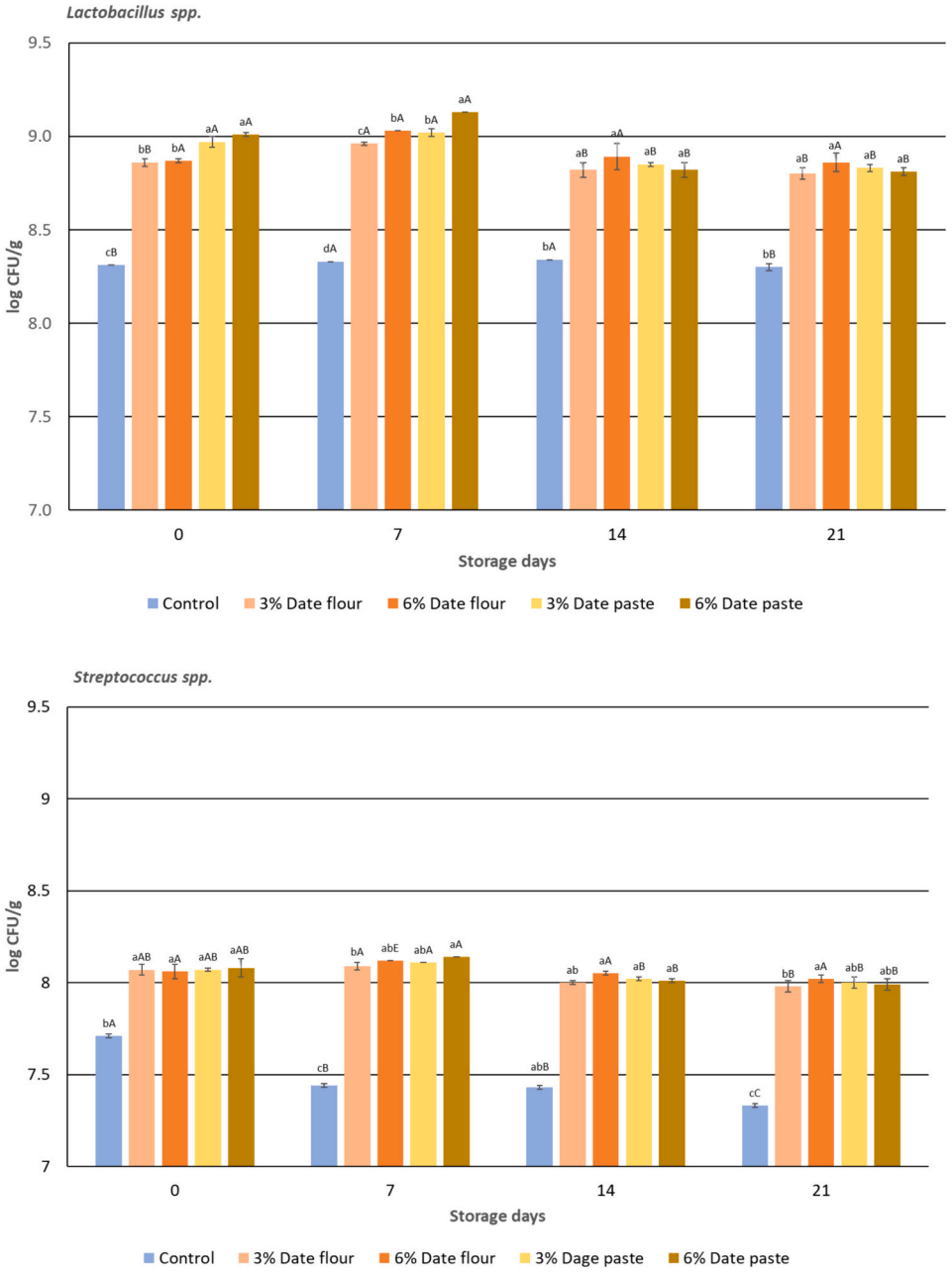


Fig. 1. Lactobacillus spp. and Streptococcus spp. counts in fortified yogurts with date coproducts during 21 days of refrigerated storage-For the same time, different lowercase letters (a-c) indicate significant differences ($p < 0.05$) between yogurt formulations. For the same formulation, different uppercase letters (A-E) indicate significant differences ($p < 0.05$) between storage times.

increase), followed by yogurts with 6% date flour (15.5% increase) and 3% date flour (11.5% increase), obtaining the lower lactic acid increase (8.9%) in control yogurts. This pattern aligns with the trends observed in the viable cell counts of starter bacteria during refrigerated storage (see Fig. 1), as there was a clear correlation between bacterial growth and acidification. The greatest rises in lactic acid content were observed in yogurts with the highest numbers of viable starter bacteria cells. A similar behavior has been detected in yogurts added with fruits extracts that enhanced the growth of starter bacteria (Bertolino et al., 2015; Sendra et al., 2008). For citric acid, slight variations between samples due to the date coproducts addition, and no significant differences due to storage time, were observed. It has been reported that these starter bacteria do not utilize citrate, since they are a Cit^- strain (Adhikari et al., 2002). Bertolino et al. (2015) did not find changes in the citric acid concentration of yogurts added with hazelnut skins during 21 days of refrigerated storage.

3.5. Yogurts' stability during cold storage

3.5.1. Physicochemical properties

Overall, the incorporation of date coproducts into yogurt formulation affected the physicochemical properties in different ways and intensity during cold storage (Table 4). The pH and titratable acidity of yogurts were affected by both the date coproducts incorporation and storage time ($p < 0.05$). All date-added yogurts showed lower pH and higher titratable acidity values than control ($p < 0.05$). This behavior was similar throughout the storage period, with no significant variations depending on the type of coproduct incorporated (paste or flour) or its concentration (3 or 6%). In general, it could be said that the storage time reduced the pH and increased the titratable acidity of yogurts. While this

Table 4
Physicochemical properties of goat yogurts fortified with date coproducts (mean ± sd).

	Storage time (days)	Yogurt				
		0%	3% Date flour	6% Date flour	3% Date paste	6% Date paste
pH	0	4.67 ± 0.05^{aA}	4.38 ± 0.01^{bA}	4.39 ± 0.01^{bA}	4.25 ± 0.02^{cAB}	4.32 ± 0.01^{bA}
	7	4.54 ± 0.04^{aB}	4.27 ± 0.01^{bB}	4.21 ± 0.02^{bC}	4.20 ± 0.01^{bB}	4.24 ± 0.03^{bB}
	14	4.51 ± 0.01^{aB}	4.26 ± 0.01^{cB}	4.22 ± 0.01^{cC}	4.26 ± 0.03^{cAB}	4.33 ± 0.01^{bA}
	21	4.53 ± 0.02^{aB}	4.39 ± 0.03^{bA}	4.33 ± 0.03^{bB}	4.33 ± 0.06^{bA}	4.37 ± 0.05^{bA}
Acidity (°D)	0	91.50 ± 0.71^{bB}	96.50 ± 2.12^{bC}	103.50 ± 2.12^{aB}	104.50 ± 0.71^{aC}	105.50 ± 0.71^{aC}
	7	96.50 ± 0.71^{cB}	105.50 ± 0.71^{bB}	108.50 ± 0.71^{bB}	113.00 ± 1.41^{aB}	115.00 ± 0.00^{aB}
	14	105.00 ± 2.83^{bA}	115.50 ± 0.71^{aA}	119.00 ± 1.41^{aA}	114.50 ± 0.71^{aA}	119.50 ± 0.71^{aA}
	21	106.00 ± 1.41^{bA}	119.00 ± 1.41^{aA}	122.50 ± 0.71^{aA}	118.50 ± 2.12^{aA}	122.00 ± 1.41^{aA}
aw	0	0.972 ± 0.001^{aA}	0.973 ± 0.001^{aA}	0.967 ± 0.001^{bA}	0.972 ± 0.001^{aA}	0.972 ± 0.000^{aA}
	7	0.973 ± 0.001^{aA}	0.972 ± 0.001^{aA}	0.971 ± 0.001^{aA}	0.972 ± 0.000^{aA}	0.971 ± 0.000^{aA}
	14	0.974 ± 0.000^{aA}	0.972 ± 0.001^{aA}	0.973 ± 0.001^{aA}	0.972 ± 0.003^{aA}	0973 ± 0.001^{aA}
	21	0.977 ± 0.001^{aA}	0.973 ± 0.001^{bA}	0.971 ± 0.000^{bA}	0.972 ± 0.001^{bA}	0.973 ± 0.000^{bA}
Syneresis (%)	0	ND	0.27 ± 0.07^{bD}	0.48 ± 0.02^{aD}	ND	ND
	7	0.10 ± 0.02^{cC}	0.50 ± 0.02^{bC}	1.29 ± 0.02^{aBC}	ND	ND
	14	0.21 ± 0.03^{cB}	0.85 ± 0.05^{bB}	1.14 ± 0.13^{aC}	ND	ND
	21	0.43 ± 0.03^{cA}	1.05 ± 0.05^{bA}	1.43 ± 0.08^{aA}	0.11 ± 0.03^{eA}	0.25 ± 0.05^{dA}
L*	0	89.39 ± 0.34^{aA}	77.35 ± 0.61^{dAB}	75.23 ± 0.37^{dA}	86.95 ± 0.13^{bB}	84.87 ± 0.10^{cA}
	7	89.57 ± 0.08^{aA}	79.48 ± 0.45^{cA}	73.08 ± 0.37^{dBC}	85.03 ± 0.79^{bB}	84.60 ± 0.35^{bA}
	14	90.11 ± 0.24^{aA}	77.95 ± 1.45^{dA}	74.57 ± 0.96^{eAB}	87.24 ± 0.85^{bA}	83.41 ± 0.29^{cB}
	21	89.83 ± 0.26^{aA}	74.59 ± 1.47^{dB}	72.22 ± 1.10^{eC}	86.38 ± 0.39^{bAB}	82.28 ± 0.42^{cC}
a*	0	−1.32 ± 0.01^{eA}	2.67 ± 0.07^{bB}	3.11 ± 0.11^{aB}	−0.45 ± 0.09^{dC}	0.34 ± 0.02^{cB}
	7	−1.39 ± 0.04^{dA}	2.06 ± 0.11^{bB}	3.23 ± 0.06^{aB}	0.31 ± 0.14^{cA}	0.30 ± 0.10^{cB}
	14	−1.31 ± 0.05^{dA}	2.44 ± 0.31^{aB}	2.97 ± 0.21^{aB}	−0.65 ± 0.08^{cC}	0.70 ± 0.15^{bA}
	21	−1.36 ± 0.03^{dA}	3.57 ± 0.59^{aA}	4.04 ± 0.43^{aA}	−0.09 ± 0.03^{cB}	0.86 ± 0.13^{bA}
b*	0	6.04 ± 0.06^{dA}	11.12 ± 0.25^{bA}	12.49 ± 0.44^{aA}	6.27 ± 0.26^{dB}	8.15 ± 0.18^{cAB}
	7	6.09 ± 0.17^{cA}	8.17 ± 0.29^{bB}	11.38 ± 0.68^{aA}	7.38 ± 0.24^{bA}	7.83 ± 0.21^{bB}
	14	6.27 ± 0.03^{dA}	9.95 ± 0.11^{bA}	11.36 ± 0.87^{aA}	6.74 ± 0.22^{dB}	8.60 ± 0.61^{cAB}
	21	6.17 ± 0.07^{dA}	11.13 ± 1.11^{aA}	13.20 ± 1.14^{aA}	7.16 ± 0.24^{cA}	9.02 ± 0.49^{bA}
C*	0	6.18 ± 0.06^{dA}	11.44 ± 0.26^{bA}	12.87 ± 0.45^{aA}	6.28 ± 0.26^{adB}	8.15 ± 0.19^{cB}
	7	6.24 ± 0.16^{dA}	8.43 ± 0.31^{bB}	11.83 ± 0.65^{aA}	7.38 ± 0.25^{cA}	7.83 ± 0.20^{bcBC}
	14	6.41 ± 0.03^{dA}	10.25 ± 0.15^{bA}	11.75 ± 0.82^{aA}	6.77 ± 0.22^{dB}	8.63 ± 0.61^{cAB}
	21	6.32 ± 0.06^{dA}	11.69 ± 1.23^{bA}	13.80 ± 1.21^{aA}	7.16 ± 0.24^{dA}	9.06 ± 0.50^{cA}
H*	0	102.34 ± 0.19^{aA}	76.47 ± 0.14^{dA}	76.04 ± 0.16^{dA}	94.08 ± 0.91^{bA}	87.60 ± 0.17^{cA}
	7	102.82 ± 0.37^{aA}	75.86 ± 0.34^{cA}	74.09 ± 0.84c^{aAB}	87.57 ± 0.99^{bB}	87.78 ± 0.80^{bA}
	14	101.85 ± 0.14^{aA}	76.24 ± 1.66^{dA}	75.27 ± 1.81^{dAB}	95.52 ± 0.80^{bA}	85.40 ± 0.77^{cB}
	21	102.44 ± 0.42^{aA}	72.28 ± 1.41^{dB}	72.97 ± 0.33^{dB}	90.77 ± 0.30^{bB}	84.56 ± 0.50^{cB}
WI[1]	0	87.72 ± 0.26^{aA}	74.63 ± 0.56^{dB}	72.09 ± 0.31^{eA}	85.51 ± 0.10^{bA}	82.82 ± 0.17^{cA}
	7	87.85 ± 0.12^{aA}	77.81 ± 0.53^{cA}	70.59 ± 0.20^{dAB}	83.30 ± 0.80^{bB}	82.72 ± 0.23^{bA}
	14	88.21 ± 0.19^{aA}	75.68 ± 1.37^{dAB}	71.98 ± 0.68^{eA}	85.55 ± 0.68^{bA}	81.29 ± 0.14^{cB}
	21	88.03 ± 0.19^{aA}	72.03 ± 1.85^{dC}	68.98 ± 1.50^{eB}	84.61 ± 0.44^{bAB}	80.09 ± 0.57^{cC}

a-e Different lowercase letters in the same row indicate significant differences ($p < 0.05$) due to date addition.
A-D Different uppercase letters in the same column indicate significant differences ($p < 0.05$) due to storage time.
ND: Not detected.
[1] WI: whitness index.

holds true for titratable acidity across all samples, the pattern differs for pH, particularly evident in yogurts containing dates towards the end of storage where the pH values increased. Among the samples, control yogurts and those with 3% date paste showed the least significant rise in lactic acid content over the 21-day storage period, while yogurts with added date flour, regardless of concentration, exhibited the highest increase. The increase in the titratable acidity and the pH decrease of control and date-added yogurts during cold storage could be due to the further metabolic activities brought about by the starter cultures during storage that hydrolyze lactose into lactic acid and dietary fiber (from date coproducts) into uronic acids, resulting in a decrease of the pH values and increasing the acidity during storage (Almusallam et al., 2021; Gaspar, Carvalho, Vinga, Santos, & Neves, 2013). Bacterial growth is uncoupled from acidification and under refrigerated conditions, bacteria continue to degrade lactose although at a much slower rate than at the optimum temperature for thermophilic bacteria (Brodziak, Król, Barłowska, Teter, & Florek, 2020). All yogurt samples during the whole storage time showed acidity values ranging from 91 to 125°D which are within the minimum recommended standard range (60–150°D) by the Codex Alimentarius Commission (Codex, 2015) for

fermented dairy products. A similar behavior of pH and acidity was reported in yogurts fortified with natural extracts such as jujube pulp (Feng et al., 2019), red ginseng (Jung et al., 2016), date palm spikelets extracts (Almusallam et al., 2021) and, beetroot purée, strawberry juice or pomegranate juice (Basiony, Saleh, Hassabo, & AL-Fargah, A., 2023).

Water activity (aw) represents the availability of the water contained in the product as the higher the aw index, the faster microorganisms can growth. In the case of fermented dairy products, the pH of the product contributes to the control of the development of spoilage microorganisms without affecting starter cultures (Godlewska, 2012). The water activity of yogurts was only affected by the addition of date coproducts but not for the storage time. At time 0, yogurts added with 6% date flour showed aw values lower ($p < 0.05$) than the rest, which could be related with the high water holding capacity of date flour and its sugar content (Muñoz-Bas et al., 2023). At the end of storage, all date-added yogurts showed aw values lower than control ($p < 0.05$). Water activity values of all samples over the storage time were in the range of the values described as normal for goat yogurts (0.97–0.98). Some authors have found that aw values in yogurts increased significantly with the storage time (Brodziak et al., 2020), however other authors reported that these values remained constant (Dos Santos et al., 2018). In this case, although the aw values increased in some samples, this increase did not result being statistically significant ($p > 0.05$).

Syneresis is a relevant physical parameter of yogurt that can affect its storage stability and consumer acceptability given that whey separation on the yogurt surface may negatively influence the yogurt's shelf life and consumer acceptability. The lower the syneresis, the higher the yogurt stability (Kiros, Seifu, Bultosa, & Solomon, 2016). The syneresis of fresh yogurt was affected by both, the addition of date coproducts and the storage time ($p < 0.05$). Yogurts added with date flour showed the highest syneresis through the storage period, being these values greater at higher date flour concentrations ($p < 0.05$). On the contrary, yogurts added with date paste showed the lowest syneresis over the storage ($p < 0.05$), except at time 0, where syneresis was not detected in these samples or in the control. It should be highlighted that the addition of date paste was able to keep the syneresis values of the yogurts undetectable up to 14 days of storage, while in the control samples, detectable syneresis values were already obtained after 7 days. These results suggest that date paste can reduce the syneresis changes during cold storage of goat milk yogurts and hence maintain their physical quality attributes. A similar behavior has been reported in yogurts added with fruit pomaces or purées (Almusallam et al., 2021; Feng et al., 2019; Jung et al., 2016; Kwon, Bae, Seo, & Han, 2019; Rashwan et al., 2024), attributed to their high water holding capacity (WHC) caused by the polysaccharide molecules that can absorb a high amount of water and improve the rigidity of the protein-gel network. Additionally, electrostatic attraction between milk proteins and fruit polysaccharides could play a role in increasing WHC and decreasing the yogurt syneresis (Du et al., 2023; Fan et al., 2023). Furthermore, some phenolic compounds contained in these extract fruits and in the date coproducts could affect the protein coagulation network and hence the syneresis (Durmus et al., 2021). Moreover, Rashwan et al. (2024) reported that these interactions that imply hydroxyl groups and anionic groups, would form a complex with positively charged protein clusters and more hydrogen bonding in the yogurt. In the case of the addition of date flour this beneficial effect on syneresis was not shown; in fact, syneresis values increased, which may be explained by the fact that this date flour was not easily dissolved in the milk and so the potential interactions polysaccharide-water and polysacharide-proteins were not correctly established. In addition, fast acidification allows faster solubilization of colloidal calcium phosphate, dissociation of caseins and massive (disordered) protein aggregation with more time around isoelectric point of caseins making difficult the possible inter-polymeric associations. Similar behavior has been described in yogurts supplemented with citrus fiber (García-Pérez et al., 2005), grape fiber (Tseng & Zhao, 2013) and hazelnuts skins (Bertolino et al., 2015) attributed to the increase in whey separation due to the presence of insoluble polysaccharides, which prompts rearrangement of the gel matrix. All yogurts showed higher syneresis values over storage time, showing the maximum values at day 21 of storage ($p < 0.05$). The increase in the syneresis during yogurt storage is a common phenomenon observed in these dairy products, often linked to increased acidity over time. This acidity increase leads to a reduction in the net negative charges of casein micelles, affecting their colloidal stability and resulting in heightened water separation from yogurt curds, as discussed by Molaee Parvarei et al. (2021). Yogurts fortified with fruits pomaces also showed few changes in syneresis during storage (Almusallam et al., 2021; Feng et al., 2019; Jung et al., 2016; Kwon et al., 2019).

The color of foods is considered one of the most important quality parameters that significantly affects its marketability and consumer acceptance. The addition of date coproducts to yogurts caused statistically significant modifications ($p < 0.05$) (with respect to the control yogurt) in all the color properties analyzed (L*, a*, b*, C*, H* and whiteness index). Date-added yogurts showed lower lightness, hue and WI and higher color saturation and a* and b* values than control yogurts throughout cold storage ($p < 0.05$). In addition, all these changes on color properties were more intense ($p < 0.05$) in yogurts with date flour than with date paste added. Similar color changes (L*, H* and WI decrease and a*, b* and C* increase) have been reported in fortified yogurts with several natural extracts (Kwon et al., 2019; Pelaes Vital et al., 2015). These color changes would be related to the orange-brown color of the added date products (Muñoz-Bas et al., 2023), due to their content in carotenes, orange-yellow to red crystalline pigments and melanin pigments (Alam et al., 2022). In fact, regarding the hue values of yogurts, the color of the yogurts changed from lemon-yellow (control) towards yellow-orangish (yogurts with date paste) and yellow-orange (yogurts with date flour) (IRANOR, 1981). The color of date-added yogurts changed throughout the storage period ($p < 0.05$) in contrast with control yogurt, whose color properties were not modified ($p > 0.05$) during 21 days of cold storage. It is noteworthy that these changes in color properties during storage didn't follow a clear trend and, in most of the cases, the variations were slight, being lower than 2–3 units. It could be said that after 21 days of refrigerated storage, date-added yogurts showed lower hue values and whiteness index than at the beginning (day 0). In any case, these changes during storage could be related to the acidity development and modifications of yogurt's three-dimensional structure that promote the release of these pigments of date products from the yogurt matrix becoming more sensible to chemical, enzymatic and microbial degradations or transformations. Relevant color changes during cold storage have been reported in yogurts added with extracts from red fruits that were attributed to the changes in the color of some bioactive compounds (e.g. anthocyanins or betalains) caused by the pH decrease (Basiony et al., 2023; Durmus et al., 2021; Rashwan et al., 2024).

3.5.2. *Mechanical properties (Texture)*

Table 5 shows the results of texture parameters, including firmness, consistency, cohesiveness, and viscosity index of all yogurts during 21 days of cold storage. In general, the addition of date coproducts increased yogurt firmness, consistency and cohesiveness, and decreased the viscosity index, being this effect higher in yogurt added with date flour ($p < 0.05$). However, the evolution of these texture parameters during refrigerated storage didn't follow a clear pattern with slight modifications over storage. At time 0 and 21, yogurts incorporated with date flour displayed higher firmness and consistency but lower viscosity index that the rest of the yogurts ($p < 0.05$). Even though the cohesiveness of yogurts was not influenced by the addition of date coproducts at time 0 ($p > 0.05$), at the end of storage, yogurts with date flour showed the highest values ($p < 0.05$). It could be said that the addition of date flour induced stronger texture modifications in yogurts than the addition of date paste. Firmness and consistency of yogurts have been previously related to the total solid content that provides a consistent and stable structure of yogurt (Wang et al., 2023) besides

Table 5
Textural properties of goat yogurts fortified with date coproducts (mean ± sd).

	Storage time (days)	Yogurt				
		Control	3% Date flour	6% Date flour	3% Date paste	6% Date paste
Firmness (N)	0	0.06 ± 0.01^{ab}	0.11 ± 0.01^{bAB}	0.10 ± 0.02^{bAB}	0.05 ± 0.01^{aA}	0.06 ± 0.03^{aB}
	7	0.06 ± 0.00^{a}	0.10 ± 0.01^{bcAB}	0.12 ± 0.02^{cB}	0.07 ± 0.02^{abB}	0.07 ± 0.02^{abB}
	14	0.05 ± 0.01^{a}	0.08 ± 0.01^{abA}	0.10 ± 0.01^{bA}	0.06 ± 0.01^{abB}	0.08 ± 0.03^{abB}
	21	0.05 ± 0.01^{a}	0.12 ± 0.02^{bB}	0.09 ± 0.01^{bA}	0.04 ± 0.01^{aA}	0.05 ± 0.01^{aA}
Consistency (N.s)	0	0.78 ± 0.19^{aB}	1.84 ± 0.49^{bB}	1.30 ± 0.51^{abB}	0.83 ± 0.16^{aB}	0.81 ± 0.06^{aC}
	7	0.56 ± 0.15^{abAB}	1.21 ± 0.21^{bcAB}	1.55 ± 0.46^{cC}	0.40 ± 0.15^{aB}	0.53 ± 0.14^{abAB}
	14	0.43 ± 0.09^{aA}	1.00 ± 0.16^{bA}	1.19 ± 0.25^{bB}	0.58 ± 0.06^{aAB}	0.62 ± 0.05^{aBC}
	21	0.37 ± 0.08^{aA}	1.32 ± 0.04^{bAB}	1.24 ± 0.07^{bA}	0.35 ± 0.03^{aA}	0.33 ± 0.03^{aA}
Cohesiveness (N)	0	-0.02 ± 0.01	-0.03 ± 0.01B	-0.03 ± 0.03^{B}	-0.04 ± 0.02^{A}	-0.03 ± 0.01^{A}
	7	-0.02 ± 0.02^{b}	-0.05 ± 0.00^{aA}	-0.06 ± 0.00^{aA}	-0.02 ± 0.01^{bB}	-0.03 ± 0.00^{bB}
	14	-0.02 ± 0.02^{c}	-0.04 ± 0.01^{bAB}	-0.06 ± 0.03^{aA}	-0.02 ± 0.00^{cB}	-0.02 ± 0.01^{cB}
	21	-0.02 ± 0.01^{b}	-0.05 ± 0.01^{aA}	-0.05 ± 0.01^{aA}	-0.02 ± 0.01^{bB}	-0.02 ± 0.01^{bC}
Index of Viscosity (N.s)	0	0.85 ± 0.05^{dC}	-0.17 ± 0.05^{aA}	-0.15 ± 0.03^{aA}	-0.05 ± 0.02^{bA}	-0.01 ± 0.01^{bA}
	7	0.51 ± 0.01^{cB}	-0.05 ± 0.01^{abB}	-0.06 ± 0.02^{aB}	-0.02 ± 0.03^{bB}	-0.02 ± 0.02^{bB}
	14	0.51 ± 0.02^{bB}	$(-)0.03 \pm 0.01^{aB}$	-0.03 ± 0.01^{aB}	-0.02 ± 0.01^{aB}	-0.03 ± 0.01^{aB}
	21	0.04 ± 0.01^{bA}	$(-)0.07 \pm 0.01^{aB}$	-0.04 ± 0.01^{bB}	-0.02 ± 0.01^{bB}	-0.03 ± 0.00^{bB}

a-d Different lowercase letters in the same row indicate significant differences ($p < 0.05$) due to date addition.
A-C Different uppercase litters in the same column indicate significant differences ($p < 0.05$) due to storage time.

other components or added ingredients such as phenolic compounds and polysaccharides that could act helping to the stabilization of the yogurt gel (Rashwan et al., 2022; Rashwan et al., 2024). Yogurt' cohesiveness is an indicator of the force needed to release the yogurt stuck to the spoon or mouth upon eating yogurt (Wang et al., 2019). Generally, yogurt viscosity has been related to the interaction between water and macromolecules such as proteins and polysaccharides (Rashwan et al., 2022). Several authors have reported improvement in yogurt texture due to the addition of fruit extracts (Fan et al., 2023; Du et al., 2023; Rashwan et al., 2024). In addition, Harper, Dobson, Morris, and Moggré (2022) highlighted that the lactic acid bacteria used in yogurt elaboration could produce exopolysaccharides which can aggregate with milk caseins, resulting in the improvement of yogurt texture.

3.5.3. Microbiological analysis

Starter culture lactic acid bacteria (*Lactobacillus* spp. and *Streptococcus* spp.) counts of the control and date-added yogurts during 21 days of refrigerated storage are shown in Fig. 1. The addition of date coproducts to yogurt did not affect the survival of the starter strains because after 21 days of storage, the viable cell numbers of both strains in all yogurts were higher than the minimum legally required in yogurt manufacture by the Codex Alimentarius (10^7 CFU/g). In particular, *Lactobacillus* spp. viable cell numbers in yogurts with date paste (3 and 6%), reached a mean value of 8.92 log10 CFU/g, higher than yogurts formulated with date flour (3 and 6%) (8.88 log10 CFU/g) and, than in control (8.33 log10 CFU/g). A similar trend was observed for the *Streptococcus* spp. viable cell numbers (7.45 log10 CFU/g in control, 8.04 log10 CFU/g in date flour added yogurts and, 8.06 log10 CFU/g in date paste added yogurts). The addition of date coproducts (both date paste and flour) enhanced the growth and stability of the starter culture of yogurts potentially enhancing the probiotic capacity of yogurts supplemented with dates. As previously mentioned, the sugar content in these date coproducts along with their content in other micronutrients such as minerals and vitamins may act as promoting agents for yogurts starter culture growth and acidification. Overall, the viability of the starter culture (*Lactobacillus* and *Streptococcus* strains) decreased slightly (lower than 0.4 CFU/g) during refrigerated storage, although this reduction was not always significant. The decrease of lactic acid bacteria viable cell numbers during storage is likely due to the acidity increase and pH reduction in yogurt during refrigerated storage (Du et al., 2023). A similar reduction in starter culture viable cell numbers during the storage of fortified yogurts with fruit extracts has been previously reported (Jaster et al., 2018; Mohamed Ahmed et al., 2021).

Enterobacteriaceae were not detected in any yogurt samples during refrigerated storage. Molds and yeasts were not detected in control yogurts and yogurts with date flour added (both concentrations 3 and 6%) during refrigerated storage. However, molds and yeasts were detected in date paste added yogurts (3 and 6%) both freshly processed and during refrigerated storage (data not shown), with mean counts of 2.15 log10 CFU/g and, with no significant differences between concentrations and storage times. Taking into account that neither molds nor yeasts were detected in control or date flour added yogurts, their presence in date paste added yogurts can be attributed to the date paste itself. In fresh dates, as well as other fresh fruits that retain moisture, molds and yeasts can be present although rarely cause problems in such foods because they grow at a slower rate in comparison with bacteria. They were not found in date flour because the heat treatment applied during its processing was enough to inactivate them. For future applications at industrial scale, date paste should be pasteurized either separately or together with the milk to ensure yogurt safety. Basiony et al. (2023) also found molds and yeasts growth in yogurts added with pomegranate, strawberry juices and red beetroot puree.

3.6. Sensory evaluation of yogurts

The results of the consumer acceptance of yogurts are shown in Table 6. The fortification of yogurts with the date coproducts was associated with a significant effect ($p < 0.05$) on color, taste, granularity and overall acceptance aligned with the corresponding instrumental analysis of color as described in section 3.5.1. On the contrary, acidity, sweetness, odor and firmness were unaffected ($p > 0.05$) by date coproducts addition. It is worth mentioning that despite the increase in titratable acidity values and lactic acid contents, there was no corresponding impact on the sensory perception of acidity. For all the parameters analyzed, all the yogurts scored higher than the central value of the scale (5, neither like nor dislike), except for the granularity in the 6% date flour yogurts (4.7). The addition of date flour (at both concentrations) led to a more brownish (see Table 4) and more granular texture, decreasing the score of both attributes ($p < 0.05$), and changes in the expected whiteness of yogurt were negatively assessed by panelists. The granularity could be associated with the insolubility of one part of the date flour added, which has also been previously reported by other authors in yogurts incorporated with vegetable extracts rich in dietary fiber (Darwish, El-Deeb, & Elgindy, 2018; Jrad et al., 2022; Sendra et al., 2008). The addition of 6% date flour decreased ($p < 0.05$) the score for taste. Based on the comments received, consumers preferred control yogurts and date paste added yogurts (3 and 6%) to yogurts with date flour (3 and 6%). This preference can possibly be explained by the

Table 6
Results of sensory evaluation (color, taste, odor, sweetness, acidity, granularity and firmness) and overall acceptance of yogurts fortified with date coproducts (mean ± sd).

Yogurt	Sensory attribute							
	Color	Taste	Odor	Sweetness	Acidity	Granularity	Firmness	Overall acceptance
Control	8.0 ± 1.0^{a}	6.5 ± 1.4^{a}	7.1 ± 1.4^{a}	5.8 ± 1.7^{a}	6.4 ± 1.5^{a}	7.0 ± 1.9^{a}	7.0 ± 2.2^{a}	7.6 ± 1.4^{a}
3% Date paste	7.4 ± 1.2^{a}	5.9 ± 1.7^{ab}	7.0 ± 1.2^{a}	5.4 ± 1.9^{a}	5.6 ± 1.1^{a}	6.7 ± 1.9^{a}	7.0 ± 2.2^{a}	7.1 ± 1.3^{ab}
6% Date paste	5.9 ± 1.7^{b}	6.2 ± 1.9^{ab}	7.1 ± 1.4^{a}	5.6 ± 2.2^{a}	6.2 ± 1.1^{a}	5.8 ± 1.0^{ab}	7.2 ± 2.6^{a}	7.1 ± 1.9^{ab}
3% Date flour	6.0 ± 1.8^{b}	5.2 ± 1.6^{ab}	6.5 ± 1.6^{a}	5.0 ± 1.6^{a}	5.2 ± 1.7^{a}	5.1 ± 1.5^{b}	7.1 ± 2.4^{a}	6.1 ± 1.7^{b}
6% Date flour	5.6 ± 2.2^{b}	5.1 ± 1.9^{b}	6.2 ± 1.6^{a}	5.5 ± 1.9^{a}	5.6 ± 1.9^{a}	4.7 ± 0.9^{b}	6.9 ± 2.1^{a}	6.2 ± 1.9^{b}

a-b Different letters in the same column indicate significant differences ($p < 0.05$) between yogurt formulation.

increased granularity and color modifications registered in yogurt formulated with date flour. However, it can be argued that yogurts fortified with date coproducts were well accepted overall (all of them received scores >5 for overall acceptance), in contrast to the low acceptance of other yogurts fortified with vegetable extracts as found in the literature (Bertolino et al., 2015; Hashim, Khalil, & Afifi, 2009; Tseng & Zhao, 2013).

4. Conclusions

Date paste and date flour, derived from the valorization of date byproducts, serve as functional ingredients to fortify goat milk yogurts. Although their addition accelerates the acidification process, the gelation of the milk proteins was not prevented, resulting in a final product with the characteristic yogurt texture. Date-added yogurts exhibit higher levels of K, Mn, Cu, and Zn, along with increased glucose and lactic acid content compared to the control. In addition, both ingredients boost the growth and stability of the yogurt starter culture, enhancing the probiotic potential of date-added yogurts. In terms of physicochemical properties, the addition of date flour (at both concentrations) induces more pronounced modifications (texture, color and syneresis) in yogurts compared to the addition of date paste. During storage, date paste reduced the syneresis of goat milk yogurts maintaining their physicochemical quality. Although all date-added yogurts were, in general, well accepted by consumers (all of them received scores >5 for overall acceptance), the yogurts with date paste (3% and 6%) were preferred over those with date flour, because its addition (date flour) led to a more brownish and more granular texture in the yogurts, which ultimately decreased the score for both attributes.

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CRediT authorship contribution statement

Nuria Muñoz-Tebar: Visualization, Methodology, Formal analysis. **Clara Muñoz-Bas:** Writing – original draft, Methodology, Formal analysis. **Manuel Viuda-Martos:** Visualization, Methodology, Conceptualization. **Estrella Sayas-Barberá:** Resources, Funding acquisition. **José Angel Pérez-Alvarez:** Writing – review & editing, Supervision, Funding acquisition. **Juana Fernández-López:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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7.6. PUBLICACIÓN 6

Application of date-coproducts for the fortification of fresh goat cheese: Effect on their nutritional, technological, physicochemical, microstructural, microbiological and sensory properties

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Application of date-coproducts for the fortification of fresh goat cheese: Effect on their nutritional, technological, physicochemical, microstructural, microbiological and sensory properties

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ABSTRACT

A sustainable approach for the fortification of fresh goat cheese, while reducing food industry waste, is adding date coproducts (as date paste) to the cheese. The addition of date paste (up to 8 %) during the fresh cheese making did not affect its technological viability with yield production (17.8–23.3 %) and pH values (5.43–5.73) within the range of traditional goat fresh cheeses. Nutritionally, the addition of date paste (DP) resulted in cheeses with better fatty acid profile (higher MUFA content) and higher mineral content (specially K, 44 % higher in cheese with 8 % DP compared to control). In reference to LAB, fortifying cheeses with DP increased their counts (Lactobacillus spp rising from 7.43 to 8.30 $\log_{10}$ CFU/g and Streptococcus spp from 6.11 to 7.45 $\log_{10}$ CFU/g), with the rise being proportional to the amount of DP added. Regarding texture, the fortification of goat cheeses with DP did not affect either their cohesion or resilience which was confirmed by the microstructure analysis showing that the DP was integrated into the protein matrix, preserving the characteristic protein-lipid network of fresh goat cheese. Sensorially, the incorporation of DP did not affect the aroma, flavor, sweetness (although fructose and lactic acid content was higher in fortified cheeses), salty and fracturability of the cheeses.

1. Introduction

According to the UN's latest report, the world population will reach 9.8 billion by 2050 and 11.2 billion by 2100 (United Nations, 2017). This growth inevitably demands an increase in food production (not only raw materials but also processed foods). In this scenario, it will be crucial to explore new food sources (e.g. insects and seaweeds), while reducing food waste by transforming co-products into value-added products. In particular, the co-products generated from the industrialization of fruits and vegetables are rich in essential nutrients and bioactive compounds. These bioactive compounds offer a range of health benefits due to their antioxidant, antimicrobial, and anti-inflammatory properties (Aqilah et al., 2023), which, in turn, are associated with the control of the development and progression of most chronic diseases like obesity, diabetes, cardiovascular diseases, neurodegenerative disorders, and cancer (Kainat et al., 2022).

The industrialization of fresh dates generates a large amount of co-products that can be transformed into high value-added ingredients for enriching various foods when they are subjected to environmentally friendly and sustainable methods (Muñoz-Bas et al., 2023, 2024; Muñoz-Tebar et al., 2023). Date paste is one of these value-added products, whose composition (rich in sugars, dietary fiber, and minerals such as K, Ca and Mg) and techno-functional properties (water holding capacity and swelling capacity) make it a suitable ingredient for the dairy industry (Muñoz-Bas et al., 2024). It has already been successfully used to fortify goat yogurts achieving an improvement in the growth and stability of the yogurt starter culture, thereby enhancing the probiotic potential of date-enriched yogurts (Muñoz-Tebar et al., 2024). Based on these results fresh goat cheese could also serve as an interesting matrix to incorporate date co-products.

Due to the nutritional content and health benefits associated with dairy consumption (Verruck et al., 2019) along with their great consumer acceptance and the wide versatility of the dairy matrix, food industry has been a pioneer in adding these value-added compounds into dairy products. In this sense, different value-added ingredients derived from fruits and their co-products (e.g. citrus, banana, dragon fruit and

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strawberries among others) are commonly obtained as a paste or even as a powder and are rich in dietary fiber and other bioactive compounds (mainly polyphenols). These value-added ingredients have been incorporated into a range of dairy products with different purposes such as stabilizer or texturizer in yogurts, as antioxidants, and as source of fiber to fulfill dietary needs, increasing satiety and regulating appetite (Dantas et al., 2022; Kowaleski et al., 2020; Wu et al., 2023). Cheese has been also reported as an excellent matrix for the incorporation of these value-added ingredients obtained from fruit co-products, improving their nutritional, functional and technological properties (Basiony et al., 2023; El-Loly et al., 2022; Mehanna et al., 2017). In the case of ice creams, these compounds have been used to reduce the high levels of fat and sugars in their formulation, while improving their physico-chemical and technological properties(de Oliveira et al., 2021; Utpott et al., 2020) as well as their organoleptic properties including sweetness, taste, color and flavor (Curti et al., 2021; Salehi, 2021). In all cases, their application in dairy products has enhanced the profile of bioactive compounds and boosted their antioxidant properties (Boyanova et al., 2022; Haghani et al., 2021).

Consumers perceive goat cheese as healthier than cheese made from cow's milk and is highly appreciated for its nutritional value, easy digestibility and low allergenic properties. Not only does it contain less lactose, making it a better option for people with lactose intolerance, but goat cheese also appeals to health-conscious consumers due to its high levels of calcium and other vitamins and minerals (Sant'Ana et al., 2013). In addition, goat milk has been associated with certain therapeutic values in human nutrition (Caleja et al., 2015; Díaz-Castro et al., 2012; Haenlein, 2004). The goat cheese market size was valued at approximately US$ 5.72 billion in 2022 and is estimated to reach around US$ 8.64 billion by 2030. Due to a variety of driving factors, the market is expected to grow at a significant rate. By region, Europe was the leading revenue generator in 2022, with fresh cheese being the most popular form (Zion, 2024). This type of cheese is traditionally made from pasteurized goat's milk, and it is a soft, pressed cheese with enzymatic coagulation characterized by a mild, acidic flavor, typical of fresh goat's milk as well as slightly salty. The aim of this work was to evaluate the effect of two concentrations (4 % and 8 %) of date paste obtained as an intermediate food ingredient from the valorization of date coproducts (Confitera cv.) on the nutritional, technological, physicochemical, microstructural, microbiological and sensory properties of fresh goat's cheese.

2. Materials and methods

2.1. Materials

The goat milk was collected from the farm of the Miguel Hernandez University (Orihuela, Alicante, Spain). The date paste (from co-products of date fruit, Confitera cv.) was obtained following the procedure described by Muñoz-Bas et al. (2024). Starter culture CHOOZIT MA4001 was purchased from Danisco (Sassenage, France) while rennet and calcium chloride ($CaCl_2$) were obtained from Arroyo Laboratories (Santander, Spain).

2.2. Cheese making

Goat milk (60 L) was pasteurized (72 °C/15 s) and separated into three batches of 20 L each (control, DP4: 4 % DP added, and DP8: 8 % DP added). The milk was cooled to 30 °C and the starter culture was added at 0.05 Danisco culture units (DCU)/L. After that, DP was incorporated to milk followed by the addition of microbial rennet 1:15,000 (20 mL/L), $CaCl_2$ (0.25 mL/L) and salt (7 g/L). Then, vats were allowed to coagulate for 45 min, cut and stirred for 10 min. Finally, cheeses were molded and stored refrigerated at 4–8 °C and relative humidity 85 % until further analysis. Fig. 1 shows the fresh cheeses developed. The yield of cheese was calculated as: [(kilograms of cheese/ kilograms of milk) x 100].

2.3. Proximate composition

Proximate composition was determined in triplicate following AOAC methods (AOAC, 2006). Moisture content was measured by drying 2 g of sample in a vacuum oven (AOAC 925.45). Protein content was estimated from the analysis of the nitrogen content through the Kjeldahl micro method, using a conversion factor of 6.38 for milk and dairy products (AOAC 981.10). The ash content was assessed by the incineration of 2 g of sample at 550 °C until the total elimination of organic matter (AOAC 923.03). The fat content was determined by the Gerber method using a butyrometer where 10 mL of sulfuric acid and 1 mL of amyl alcohol were added to 5 g of sample.

2.4. Fatty acid profile

Fatty acid profile of the cheeses was analyzed by extracting and



Fig. 1. Appearance of fresh goat cheeses fortified with different proportions of date paste.
Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

methylating the lipids. The resulting fatty acid methyl esters (FAMEs) were quantified using gas chromatography (GC) under the same conditions described by Pellegrini et al. (2018). Briefly, an autosystem chromatographer (Perkin Elmer – Beaconsfield, UK) equipped with a VF–23 ms fused silica capillary column (30 × 0.25 mm × 0.25 μm film thickness, Varian – Middelburg, The Netherlands) and a flame ionization detector (FID) were used. The column was maintained at 60 °C for 1 min after injection, the temperature was set at 130 °C (10 °C/min), then the temperature was set at 170 °C (3 °C/min) and the last ramp at 230 °C (10 °C/min), hold during 5 min. Helium was used as a carrier gas with a column inlet pressure set at 20 psi and a split ratio of 1:20. The injection volume was 0.5 μL and the total analysis time was 32 min. The injector and detector temperatures were set at 250 °C and 270 °C, respectively. All measurements were performed in triplicate and the results were reported as g FAME/100 g of fat.

2.5. Organic acids and sugar composition

Sugars and organic acids of fresh goat cheese added with date paste were extracted using the method described by Muñoz-Bas et al. (2024). After extraction, 20 μl of each sample were injected into an HPLC (Hewlett-Packard 1100 series, Woldbronn, Germany) equipped with a Supelco Supelcogel™ C-610H column (300 mm x 7.8 mm). Orthophosphoric acid in water (0.1 % v/v) was used as the elution buffer and organic acids were quantified by measuring absorbance at 210 nm with a diode array detector (DAD G-1315 A), while sugars were detected using a refractive index detector (RID G1362A).

2.6. Mineral composition

To analyze the mineral composition of fresh goat cheese enriched with date paste, samples were lyophilized using a Freeze Dryer Alpha 2–4 (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and digested with nitric acid (67 %) and hydrogen peroxide (33 %) via microwave. Minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn) were quantified using inductively coupled plasma mass spectrometry (ICP-MS) on a Shimadzu MS-2030 (Shimadzu, Kyoto, Japan) under the same conditions used by Muñoz-Bas et al. (2023). Each cheese formulation was analyzed in triplicate and the results were expressed as mg/100 g cheese.

2.7. Physicochemical properties

The pH of cheeses was measured with a pH-meter Sension + pH31 (HACH, Spain) and water activity using a NOVASINA TH-200 hygrometer (Novasina; Lachen, Switzerland). CIELAB color coordinates were determined using a Minolta CM-700d spectrophotocolorimeter (Konica Minolta, Osaka, Japan) with a D65 illuminant and a 10° observer angle. From these coordinates, the psychophysic attributes (chroma and hue), the whiteness index (WI) and the color differences (ΔE*) respect to control cheese were calculated (Muñoz-Bas et al., 2024). All measurements were performed in triplicate.

2.8. Texture

A Texture Profile Analysis (TPA) was conducted using a TA-XT2i texturometer (Stable Micro Systems Ltd., Godalming, Surrey, UK) equipped with a 5 kg load cell and a cylindrical aluminum P/100 probe. Cheese samples were cut into 1.5 cm cubes compressed twice to 25 % of its original height at a speed of 1.3 mm/s, with a 5-second recovery time between compressions. Ten measurements were made per sample and the results were expressed as hardness (N), adhesiveness (N·seg), springiness, cohesiveness and resilience.

2.9. Confocal laser scanning microscopy (CLSM)

The microstructures of the fresh goat cheese samples were analyzed using a Leica SP5 confocal laser scanning microscopy (TCS-SPE, Leica Microsystems, Heidelberg, Germany) following the method described by Muñoz-Tebar et al. (2022) with slight modifications. Samples were cut into 95-μm-thick serial sections with an HM400 microtome (Microm, Walldorf, Germany) and soaked in fluorescence stains for around 10 min. The staining was performed using a combination of Fast Green FCF and Nile Red. Both stains were obtained from Sigma-Aldrich (Madrid, Spain) and prepared as stock solutions (1 mg/mL in deionized water for Fast green FCF and Lectin FITC and in dimethylsulfoxide (DMSO) for Nile red). These stock solutions were then diluted with deionized water and DMSO to achieve a final concentration of 0.1 mg/mL just before staining. Nile Red was excited at 488 nm, while Fast Green FCF was excited at 633 nm. The emission filters were set to 520–590 nm for Nile Red and FITC lectins, and to 660–750 nm for Fast Green FCF.

2.10. Microbiological quality

Total aerobic count, LAB, Enterobacteria and Molds and Yeasts of fresh goat cheeses incorporated with date paste was evaluated following the procedure described by Muñoz-Tebar et al. (2024). Briefly, samples were manually inoculated in duplicate on MRS agar for *Lactobacillus* spp. and M17 agar for *Streptococcus* spp., followed by incubation at 37 °C for 48 h. *Streptococcus* spp. was incubated under normal conditions, while *Lactobacillus* spp. was incubated in an anaerobic chamber using Anaerocult A (Merck, Darmstadt, Germany). For total aerobic count, enterobacteria, molds, and yeasts, Petrifilm plates (3M, Madrid, Spain) were inoculated and incubated at 37 °C for 24 h (total aerobic), 37 °C for 24 h (enterobacteria) and at 25 °C for 120 h (molds and yeasts). Plates (30–300 colony-forming units (CFU)) were manually counted expressing the results as $\log_{10}$ CFU/g of cheese.

2.11. Sensory analysis

To assess the sensory acceptability of cheese made with date paste, fifty consumers (60 % female, 40 % male, aged 18 to over 65) were recruited from the students and staff of the Orihuela Polytechnical High School at Miguel Hernández University (UMH) who were regular consumers of goat's cheese. Sensory evaluations (consumer study) were conducted in the standardized sensory laboratory at UMH, which complies with international standards (ASTM, 1986). Participants were seated in individual booths under TL 5 fluorescent lighting (Philips-Ibérica, Madrid, Spain) with an intensity of approximately 350 lx. Cheese samples (pieces of 2 × 2 cm obtained from the inner part of the cheese), each labeled with a random 3-digit code, were served in a blind, fully randomized order in transparent plastic cups. Consumers were asked to evaluate the following 9 attributes (selected based on the most appreciated by cheese consumers or those most likely to be modified by the incorporation of date paste): odor, taste, color, sweetness, salty, firmness, fracturability, granularity, and overall acceptability. A discrete 9-point hedonic scale ranging from "dislike extremely" (1) to "like extremely" (9) was used (Ramirez-Rivera et al., 2018).

2.12. Statistical analysis

Data analysis was conducted using SPSS (IBM SPSS Statistics version 26). A one-way ANOVA was applied with a 95 % confidence level to identify any significant differences between the control cheese and those added with date paste (4 % and 8 %). When significant differences were found ($p < 0.05$), Tukey's test was performed to identify specific differences between the cheese formulations. All the analysis were performed using 5 independent samples ($n = 5$) and all the assays were carried out in triplicate, except for texture analysis (10 repetitions).

3. Results and discussions

3.1. Cheese yield

Cheese yield (kg cheese/100 L milk) is a crucial parameter to evaluate the viability and efficiency of the cheese-making process and it is partially determined by the overall quality of the milk used for its production (Guo et al., 2004). The addition of DP decreased the cheese yield in a concentration-dependent manner, but all the values obtained (Control: 23.37 %; DP4: 19.83 %; DP8: 17.78 %) were within the normal range for artisanal fresh goat's cheese (Vacca et al., 2018). Cheese yield is affected by many factors including milk quality (milk composition, pasteurization, etc.) and cheese-making conditions (coagulant type, vat design, curd firmness at cutting and others manufacturing parameters) (Cipolat-Gotet et al., 2013). Given that the only difference among the 3 formulation was the DP addition, it is likely that the effect of DP has on the cheese curd formation and on its physicochemical and rheological properties was a key factor in these values.

3.2. Proximate composition

The nutritional values of cheeses depend on milk characteristics and conditions occurring during their technological processing. In addition, the chemical composition of goat milk is influenced by several factors, including the breed, individual differences among goats, diet, season, stage of lactation, and environmental conditions (Guo et al., 2004). Proximate composition of goat cheeses formulated with DP is presented in Table 1, noticing that the composition of the DP added also contribute to their nutritional value. Cheeses with DP added displayed a higher moisture content and a lower protein content ($p < 0.05$) compared with control cheese while there were not differences ($p > 0.05$) for these two parameters among the two DP added cheeses (DP4 and DP8). Date paste is likely responsible for increasing moisture retention during cheese manufacturing, mainly due to its water holding capacity (Muñoz-Bas et al., 2024). On the contrary, fat and ash content were not affected ($p > 0.05$) by DP addition. Like goat milk, fat was the main component in fresh cheeses after the moisture content. For all cheeses, the levels of moisture, fat, protein and ash were within the normal range for fresh goat milk cheeses (Kawęcka & Pasternak, 2022; Masotti et al., 2012).

3.3. Lipid profile

The fatty acid profile of the control and fortified fresh goat cheeses is shown in Table 2. The addition of DP did not affect the lipid profile of cheeses, except for oleic acid (C18:1), which increased and capric acid (C10:0) that decreased ($p < 0.05$) in DP-added cheeses. This behavior could be due to the fact that oleic acid (C18:1) is the major fatty acid in date fruits (Ogungbenle, 2011) and capric acid (C10:0) one of the most characteristic fatty acids in goat milk (Vieitez et al., 2016). Capric acid (C10:0) was the predominant short chain fatty acids (SCFA) in all cheeses, with a value exceeding 7 % of the total fatty acids in control cheese, consistent with the results reported by other authors (Paszczyk & Łuczyńska, 2020). The major fatty acids in all cheeses were palmitic acid (C16:0) followed by oleic acid (C18:1), representing the sum of more than 50 % of the total fatty acids (56.1–58.5 %). It has been reported that proportions of specific groups of fatty acids in foods are considered particularly important from a nutritional point of view (Paszczyk & Łuczyńska, 2020). Saturated fatty acid (SFA) was the main fraction (ranging from 63.4 to 64.0 %) followed by monounsaturated fatty acids (MUFA; ranging from 27.2 to 27.7 %) and a small proportion of polyunsaturated fatty acids (PUFA; ranging from 5.1 to 5.3 %). It is important to highlight that while the specific content of individual PUFAs (C18:2 and C18:3) did not differ significantly ($p > 0.05$) between the cheeses, the overall PUFA fraction was ($p < 0.05$), with the DP-added cheeses (DP4 and DP8) showing slightly higher PUFA content than the control (Table 2). Traditionally, the high levels of SFA in dairy products have been linked to negative health effects and an increased risk of conditions such as obesity, diabetes and cardiovascular diseases, among others. However, recent findings suggest that this relationship may be less straightforward than previously assumed. Other nutrients and various associated factors appear to significantly influence the lipoprotein metabolism, which plays a key role in the development of these diseases (Gómez-Cortés et al., 2018; Paszczyk & Łuczyńska, 2020). On the other hand, cheeses also contain other lipid fractions that are related to positive health aspects. For instance, SCFAs are important in promoting human health (Gómez-Cortés et al., 2018; Hanuš et al., 2018), while MUFAs (oleic acid) exhibit anti-cancer and anti-atherogenic properties, making them beneficial for daily consumption (Hanuš et al., 2018), and PUFAs provides protection against several diseases (Kapoor et al., 2021).

Table 1
Proximate composition (mg/100 g cheese) of goat cheeses formulated with date paste (DP).

Sample	% Protein	% Fat	% Ash	% Moisture
Control	13.52 ± 0.16[a]	22.87 ± 1.50[a]	2.33 ± 0.06[a]	60.23 ± 0.66[B]
DP4	12.42 ± 0.25[B]	22.29 ± 0.57[a]	2.19 ± 0.10[a]	63.07 ± 0.43[a]
DP8	12.65 ± 0.42[B]	22.23 ± 0.72[a]	2.13 ± 0.15[a]	62.94 ± 0.60[a]
p-value	0.009	0.715	0.155	0.001

[a-b] Different letter in the same column indicate significant differences based on Tukey' test ($p < 0.05$).

Data ($n = 5$; Mean ±standard deviation). Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

Table 2
Fatty acid profile of goat cheeses formulated with date paste (DP).

Sample	Control	DP4	DP8	p-value
Caprylic acid (C8:0)	1.72 ± 0.18[a]	1.43 ± 0.08[a]	1.43 ± 0.14[a]	0.200
Capric acid (C10:0)	7.65 ± 0.35[a]	6.78 ± 0.15[b]	6.83 ± 0.48[b]	0.046
Lauric acid (C12:0)	4.10 ± 0.03[a]	3.88 ± 0.01[a]	3.88 ± 0.14[a]	0.119
Myristic acid (C14:0)	9.60 ± 0.09[a]	9.53 ± 0.03[a]	9.51 ± 0.10[a]	0.51
Palmitic acid (C16:0)	31.09 ± 0.28[a]	31.73 ± 0.09[a]	31.63 ± 0.30[a]	0.141
Palmitoleic acid (C16:1)	1.19 ± 0.36[a]	0.96 ± 0.01[a]	0.95 ± 0.01[a]	0.506
Stearic acid (C18:0)	9.85 ± 0.06[a]	10.11 ± 0.06[a]	10.11 ± 0.17[a]	0.161
Oleic acid (C18:1)	25.97 ± 0.05[b]	26.73 ± 0.02[a]	26.63 ± 0.31[a]	0.046
Linoleic acid (C18:2, Ω−6)	3.87 ± 0.03[a]	4.02 ± 0.00[a]	4.01 ± 0.04[a]	0.056
α-linolenic acid (C18:3, Ω−3)	1.20 ± 0.02[a]	1.26 ± 0.01[a]	1.29 ± 0.01[a]	0.630
∑ SFA	64.03 ± 0.25[a]	63.46 ± 0.05[a]	63.40 ± 0.39[a]	0.171
∑ MUFA	27.16 ± 0.30[a]	27.69 ± 0.03[a]	27.58 ± 0.33[a]	0.238
∑ PUFA	5.07 ± 0.06 [b]	5.28 ± 0.01[a]	5.30 ± 0.05[a]	0.035

[a-b] Different letters in the same row indicate significant differences based on Tukey' test ($p < 0.05$).

Data ($n = 5$; Mean ±standard deviation). SFA: saturated fatty acids; MUFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids. Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

3.4. Sugar and organic acid composition

Table 3 shows the sugar and organic acids concentration of the fresh goat cheese enriched with date paste. The main sugar found in all the

Table 3
Sugars (lactose and fructose) and organic acids (lactic and citric acids) content (mg/g) of fresh goat cheeses with date paste (DP).

Sample	Lactose	Fructose	Citric acid	Lactic acid
Control	27.35 ± 0.14[a]	ND[c]	3.64 ± 0.07[a]	8.64 ± 0.19[b]
DP4	27.33 ± 0.24[a]	1.81 ± 0.02[b]	3.56 ± 0.10[a,b]	12.87 ± 0.02[a]
DP8	27.97 ± 0.13[a]	3.66 ± 0.04[a]	3.31 ± 0.01[b]	14.50 ± 1.36[a]
p-value	0.062	0.000	0.030	0.011

[a-c] Different letters in the same column indicate significant differences based on Tukey' test ($p < 0.05$).

ND: not detected; Data ($n = 5$; Mean ±standard deviation). Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

cheeses was lactose, with concentrations ranging from 27.3 to 28.0 mg/g, showing no significant differences ($p > 0.05$) between the control cheese and those formulated with DP. Fructose was only detected in DP-added cheeses, suggesting that its presence is due to the incorporation of this date coproduct, which contains 137.72 mg/g of fructose (Muñoz-Bas et al., 2024). Furthermore, the fructose content increased proportionally with the amount of date paste added to the cheeses (DP4: 1.8 mg/g vs. DP8: 3.7 mg/g). As expected, lactic acid was the major organic acid followed by citric acid (Table 3). The content of lactic acid increase with the incorporation of DP varying from 8.6 to 14.5 mg/g, with the highest value found in DP8 cheese. This increase in lactic acid content caused by the incorporation of DP is related to the behavior observed in LAB counts (point 3.9), which were higher in DP-added cheeses. On the other hand, citric acid content slightly decreased with the addition of date paste (from 3.6 to 3.3 mg/g), being proportional to the increase in date concentration in the cheeses. Moreira et al. (2020) reported a similar profile of organic acids (lactic and citric acid as the major) and sugars (mainly lactose) in goat cheese at the beginning of the ripening period (Day 1), being comparable to a fresh cheese. Therefore, it could be said that the values and profile of sugars and organic acids for all the cheeses fall within the range reported in the literature for goat cheese.

3.5. Mineral composition

The fortification of goat cheeses with DP significantly increased ($p < 0.05$) the content of all analyzed minerals (Ca, Cu, Fe, K, Mg, Mn, Na and Zn) compared to the control (Table 4). The major minerals found in all goat cheeses were Na, Ca, K and Mg and the high Na content is mainly due to the salt addition during cheese making. Among all these macroelements, K showed the greatest increased the most (44 % in cheese with 8 % DP compared to control) likely due to the incorporation of date paste since this co-product, obtained from dates at Tamar stage of the Confitera cv., is an excellent source of K (658 mg/100 g; Muñoz-Bas et al. (2024)). In addition, this date paste is also rich in Mg, Ca and Na (Muñoz-Bas et al., 2024) which could also contribute to the increases of these minerals in DP-fortified cheeses. Calcium is another essential microelement in cheese making (and it is often added during the process) because it binds strongly to casein in milk. However, a significant proportion of calcium is solubilized by the lactic acid produced during fermentation and is removed during the draining (Guo et al., 2004). Once the cheese production is complete, both Ca and Mg have been reported as the main macroelements linked to the solid phase of the cheese, which suggests low mobility and minimal loss during storage (Herman-Lara et al., 2019). Regarding the microelements, the significant increase in Zn, Mn and Cu in the cheeses due to DP incorporation is related to the content of these minerals in the date paste (Muñoz-Bas et al., 2024). The highest increase was observed in the Cu (from 0.01 to 0.06 mg/100 g cheese) and Mn (0.07 vs. 0.12 mg/100 g cheese) contents while Zn showed the lower increase (from 1.43 to 1.98 mg/ 100 g cheese). Several factors that can influence the mineral content of goat cheeses, leading to wide variability in reported data. However, our results are within the ranges reported by other authors (Herman-Lara et al., 2019; Moreno-Rojas et al., 2010). Based on the recommended dietary allowance (RDA) or, where the RDA is unavailable, the adequate intake (AI) for minerals in an adult male (30–50 years) as defined by the EFSA (EFSA, 2024), it has been estimated that consuming 100 g of DP8 cheese would provide the following daily mineral values: 54 % of the AI of Ca, 38 % of the RDA of P, 36 % of the AI of Na, 18 % of the RDA of Zn, 16 % of the AI of Fe, 5 % of the RDA of Mg, 5 % of the AI of Mn and 3 % of the AI of K.

3.6. Pysicochemical properties

Physicochemical parameters of control and DP-fortified cheeses are showed in Table 5. It can be observed that all the cheeses showed pH values within the usual range for fresh cheeses (5.3–6.0). In general, cheese is more acidic (lower pH) than milk (6.5–6.7 in goat milk), and this increased acidity has a positive impact on several characteristics in cheese, such as food safety, texture and flavor. The addition of DP caused a slight pH decrease ($p < 0.05$) of cheese compared to the control (from 5.73 to 5.57 in cheese with 8 % DP). This decrease in pH has also been reported by other authors in cheese incorporated with fruit extracts rich in phenolic compounds (Ferreira et al., 2024; Jeong et al., 2017; Soliman et al., 2022). Water activity (Aw) is an important biophysical factor in cheese making, as it involves a transformation of milk, a perishable liquid, into a semi-solid product with a limited shelf-life, depending on the type of cheese. In fresh cheeses, the added salt and the draining process have the greatest influence on their Aw. In this case, there were no significant differences ($p > 0.05$) in water activity between control and DP-added cheeses (Table 5), with all Aw values within the normal range for fresh cheeses (0.941–0.961; Trmčić et al. (2017)).It is important to note that although DP-added cheeses showed higher moisture content than control (Table 1), their water activity values were similar,

Table 4
Mineral profile (mg/100 g) of goat cheeses formulated with date paste (DP).

Sample	Control	DP4	DP8	p-value
Ca	503.97 ± 2.94[c]	517.82 ± 6.43[b]	536.86 ± 4.51[a]	0.000
Cu	0.01 ± 0.00[c]	0.03 ± 0.01[b]	0.06 ± 0.01[a]	0.000
Fe	0.41 ± 0.01[b]	0.55 ± 0.04[a]	0.63 ± 0.05[a]	0.001
K	98.81 ± 5.06[b]	105.87 ± 1.51[b]	141.00 ± 3.90[a]	0.000
Mg	17.68 ± 0.11[b]	17.71 ± 0.07[b]	22.37 ± 0.56[a]	0.000
Mn	0.07 ± 0.00[b]	0.09 ± 0.01[b]	0.12 ± 0.00[a]	0.000
Na	636.49 ± 14.25[b]	669.96 ± 17.84[b]	778.76 ± 6.63[a]	0.000
P	260.25 ± 3.80[a]	257.22 ± 1.00 [a]	263.49 ± 5.33[a]	0.214
Zn	1.43 ± 0.04[b]	1.46 ± 0.04[b]	1.98 ± 0.14[a]	0.000

[a–c] Different letters in the same row indicate significant differences based on Tukey' test ($p < 0.05$). Data ($n = 5$; Mean ±standard deviation). Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

Table 5
Physicochemical properties of goat cheeses formulated with date paste (DP).

Sample	Control	DP4	DP8	p-value
Aw	0.962 ± 0.000[a]	0.961 ± 0.006[a]	0.963 ± 0.002[a]	0.880
pH	5.73 ± 0.04[a]	5.43 ± 0.02[c]	5.57 ± 0.03[b]	0.000
L*(D65)	82.72 ± 0.72[a]	79.90 ± 3.06[a,b]	78.02 ± 3.73[b]	0.035
a*(D65)	−1.50 ± 0.05[b]	−0.57 ± 00.70[a,b]	0.02 ± 1.00[a]	0.007
b*(D65)	6.63 ± 0.18[a]	7.64 ± 1.31[a]	8.82 ± 3.16[a]	0.194
C*(D65)	6.80 ± 0.18[a]	7.69 ± 1.29[a]	8.85 ± 3.20[a]	0.236
h(D65)	102.78 ± 0.58[a]	94.91 ± 5.05[a,b]	90.90 ± 4.99[b]	0.001
WI	81.42 ± 0.70[a]	80.31 ± 0.55[a]	76.74 ± 1.54[b]	0.001
ΔE*		1.33 ± 0.52[b]	5.29 ± 1.35[a]	0.001

[a-c] Different letters in the same row indicate significant differences based on Tukey' test ($p < 0.05$). Data ($n = 5$; Mean ±standard deviation); WI: whiteness index. Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

suggesting that water in the DP-added cheeses would be likely bound to the main components of the date paste (sugars and dietary fiber; Muñoz-Bas et al. (2024)) and therefore not available to be involved in the cheese spoilage reactions.

The assessment of color changes in dairy products due to the addition of new ingredients is an important factor in the evaluation of their quality since it is closely associated with overall dairy food quality and eligibility (Lipşa et al., 2024; Nontasan et al., 2012). When it comes to cheese, color is an important trait, although studies have shown that a pleasant mouthfeel and flavor are more important than appearance with respect to overall taste (Milovanovic et al., 2020; Ritvanen et al., 2005). The results of the color properties of the fortified cheese samples are shown in Table 2 showing that yellowness (b*; + yellow/- blue) and chroma (C*; saturation) were not affected ($p > 0.05$) by DP addition. The addition of DP significantly reduced ($p < 0.05$) lightness (L*) and hue (h*; hue angle) values in cheeses, with greater decrease observed as the percentage of DP added increased. Goat cheese has been described as brighter compared with sheep or cow cheese which aligns with the findings reported in the literature (Milovanovic et al., 2020). A decrease in L* values in cheeses has also been reported by several authors due to the addition of vegetable extracts (Caleja et al., 2015; Giroux et al., 2013). Control cheeses showed a lemon-yellow hue, which shifted to a yellow-lemony hues when DP was added (IRANOR, 1981). On the other hand, DP-added cheeses exhibited higher redness values (a*; + red/- green) than control. This decrease in L* and h* values and the increase in a* values in DP-added cheeses compared with control could be attributed to the color properties of DP (L*=30.29; a*=2.51; h*=62.87; Muñoz-Bas et al., 2024). In dairy products, whiteness is often the most critical color characteristic, specifically for fresh cheeses, which generally maintain the milk's original whiteness. Fresh goat's cheeses, in particular, have the highest whiteness mainly because goat's milk has smaller fat globules and undergoes a total conversion of β-carotene (orange-yellow) into vitamin A (colorless) (Milovanovic et al., 2020). The addition of DP decreased the whiteness index in the cheeses, although it was significant only when 8 % DP was added ($p < 0.05$). The relevance of these color changes in the cheeses is also reflected in the color differences (ΔE*) between DP-added cheeses and the control cheeses. DP4 cheese had color differences lower than 3 units which is generally consider imperceptible to the human eye (Martínez et al., 2001). In contrast, DP8 cheese showed color differences higher than 5 units, indicating that its color would be noticeable different from the color of control cheeses. As expected, the incorporation of DP caused significant modifications in the color properties of the cheeses. It should be noted that the DP-fortified cheese exhibited a lemon-yellow hue, but this color was not uniform, instead particles of DP were visible, distributed unevenly both on the surface and throughout the inside of the cheeses (Fig. 1).

3.7. Texture

The texture parameters of control and fortified cheeses with DP, such as firmness, adhesiveness, springiness, cohesiveness, chewiness, and resilience are presented in Table 6. All textural parameters were affected by DP addition except cohesiveness and resilience. Cohesiveness values close to 1.0 indicate that the cheese can withstand the first compression cycle without disintegrating, while values close to 0 suggest complete disintegration (Paredes et al., 2022). In this study, all cheeses showed high cohesiveness values (> 0.85). Resilience, on the other hand, measures how well the cheese can recover its shape after the first compression cycle. Thus, it could be said that the fortification of goat cheeses with DP did not affect either their cohesion or resilience. However, firmness, adhesiveness and springiness were significantly affected ($p < 0.05$) by the concentration of DP added but without a clear tendency. Firmness showed the most noticeable effect varying from DP concentrations: it decreased with the addition of low DP concentrations (4 %) but increased with higher concentrations (8 %), which could indicate that the addition of DP affected the cheesemaking process. The pH changes in the DP-added cheese (Table 5) and its interaction with the enzymes (rennet) and starter cultures are crucial for moisture retention and curd formation (breaking down milk proteins). These factors influence the firmness, elasticity and structure of the curd, ultimately affecting the final texture of the cheese (Milovanovic et al., 2020). Springiness, which is related to the recovery of the material and its viscoelastic properties (Johnson, 2023), was only modified when DP was added at 4 % (decreased), showing control and DP8 cheeses similar values ($p > 0.05$). On the contrary, adhesiveness was only modified when DP was added at 8 % (−0.13 vs −0.04 N*seg). This parameter measures the effort necessary to overcome the attractive forces between the chesses' surface and the surfaces it comes into contact with, such as the tongue, teeth or palate. When DP was added at high concentrations (8 %), it may not have been fully integrated into the lactic matrix and as a result, the high adhesiveness of DP (due to its high sugar content, Muñoz-Bas et al. (2024) would prevail, significantly increasing the chesses' adhesiveness.

3.8. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) is commonly employed to study food protein gel systems, providing detailed microstructural information about multi-component gel foods. This technique could enhance the analysis of texture and functional properties in cheese formulated with non-dairy ingredients. The CLSM images (Fig. 2) reveal the microstructures of fresh goat milk cheese influenced by the addition of date paste. As shown in Fig. 2, fat globules (red) and minimal pore spaces (black) are dispersed throughout the protein matrices (green) in all cheeses, regardless of the presence of date paste. This study demonstrated a more compact network with fewer pores compared to the observations by Wang et al. (2023) for the same type of cheese. In the control samples, fat globules were randomly dispersed within the casein matrix, displaying a regular spherical shape without any polysaccharides present. However, large fibers from the date paste, significantly larger than the fat globules (50 μm vs. 3–4 μm), were observed. As shown in Fig. 2, the structure of the protein network did not differ with respect to the control cheese and the inclusion of the date paste did not appear to alter the microstructure of the cheese. The date paste was integrated into the protein matrix, preserving the characteristic protein-lipid network of fresh goat cheese. The enlarged details of the CLSM images of cheeses with date paste reveal fat and protein globules embedded within the date fibers, confirming their successful incorporation into the fresh goat cheese.

Table 6
Texture properties of goat cheeses formulated with date paste (DP).

Sample	Firmness (N)	Adhesiveness (N*s)	Springiness	Cohesiveness	Resilience
Control	2.23 ± 0.40[c]	−1.13 ± 0.00[a]	0.18 ± 0.05[a,b]	0.91 ± 0.05[a]	0.50 ± 0.02[a]
DP4	2.74 ± 0.13[b]	−0.70 ± 0.01[a]	0.12 ± 0.03[c]	0.85 ± 0.08[a]	0.45 ± 0.10[a]
DP8	3.16 ± 0.23[a]	−0.04 ± 0.01[b]	0.22 ± 0.03[a]	0.85 ± 0.06[a]	0.41 ± 0.03[a]
p-value	0.000	0.01	0.046	0.468	0.263

[a-c] Different letters in the same column indicate significant differences based on Tukey' test ($p < 0.05$). Data ($n = 5$; Mean ±standard deviation). Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added.

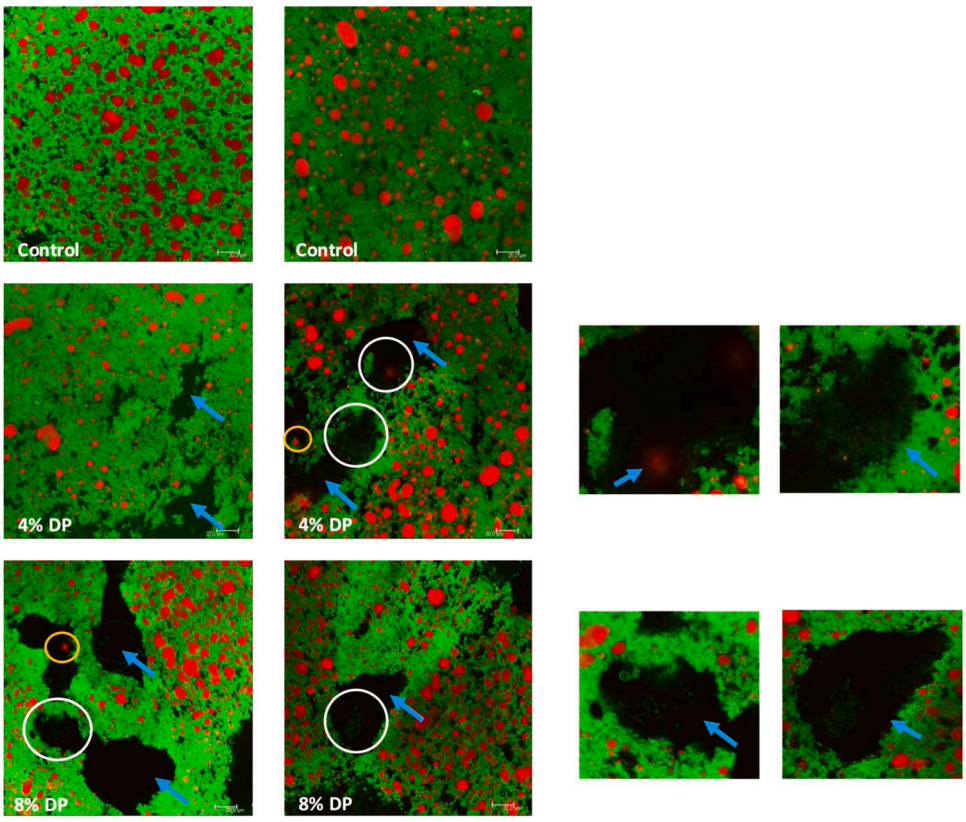


Fig. 2. Effect of incorporation of date paste on the microstructural of fresh goat cheese.
Blue arrows point to the date fibers, fat globules and proteins within the fibers. The Fast green FCF-stained protein appears green and the Nile red-stained lipid bodies appear red. Scale bars= 20 μm.

3.9. Microbiological quality

The incorporation of date paste in the cheese did not impair the development and growth of the starter strains. In fact, as the concentration of date paste increased, LAB counts were higher ($p < 0.05$) compared to control cheese, with *Lactobacillus* spp rising from 7.43 to 8.30 $\log_{10}$ CFU/g and *Streptococcus* spp increasing from 6.11 to 7.45 $\log_{10}$ CFU/g in a way dependent on the content of DP added. However, a significant reduction ($p < 0.05$) in total aerobic counts was observed in DP-fortified cheeses: from 8.05 $\log_{10}$ CFU/g in control cheeses to

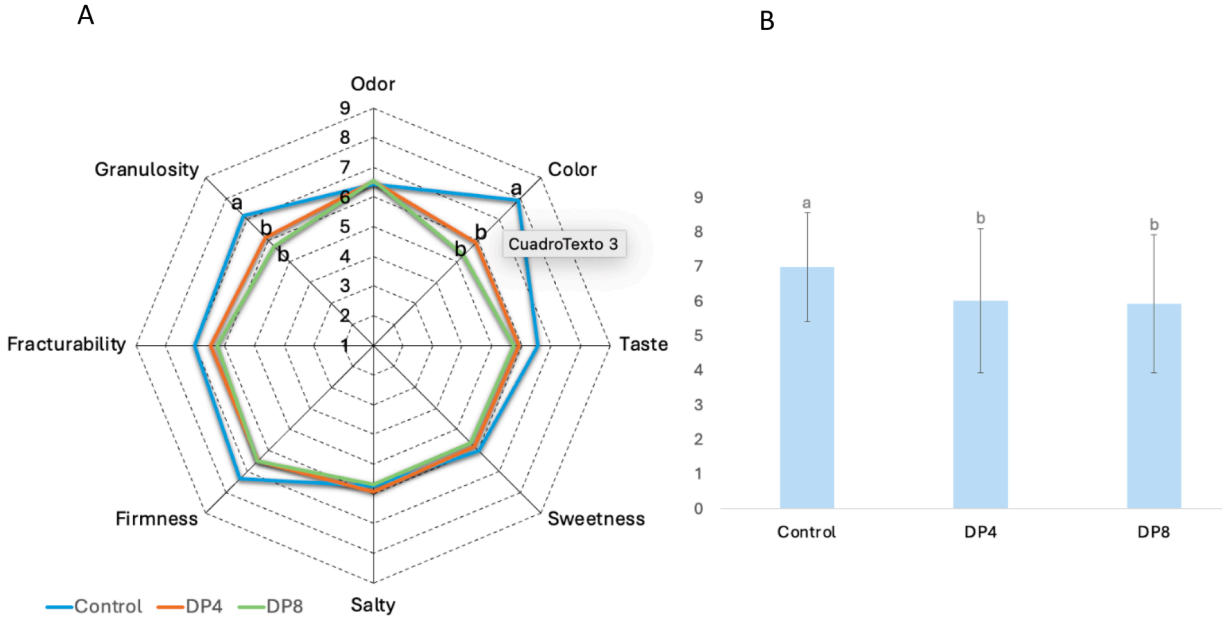


Fig. 3. Sensory evaluation (A) and overall acceptance (B) of fortified goat cheeses.
Control: cheese without date paste added; DP4: cheese with 4 % date paste added; DP8: cheese with 8 % date paste added
Data are presented as mean ± SD. Different letters indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

6.87–6.99 $\log_{10}$ CFU/g in DP-added cheeses. *Enterobacteriaceae* were not detected in any sample, and molds and yeasts were only found at low levels in DP-fortified cheeses (< 2.80 $\log_{10}$ CFU/g). These counts would indicate good sanitary practices during cheesemaking (D'amico, 2014). The presence of molds and yeasts in cheese formulated with date paste is mainly due to date paste itself, as fresh dates can harbor these microorganisms. Nevertheless, they generally develop at a slower rate than bacteria and rarely cause spoilage in such products. Despite the low levels of mold and yeast counts, it would be recommended to pasteurize the date paste, either separately or along with the milk, for future industrial-scale production. Previous studies have reported increases in LAB counts, as well as the presence of molds and yeasts in milk and dairy products enriched with fruit pulp and juices (Basiony et al., 2023; Muñoz-Tebar et al., 2024; Mwangi et al., 2023).

3.10. Sensory properties

Out of the 8 sensory attributes evaluated by the panelists (Fig. 3), significant differences ($p < 0.05$) were only detected between the control and DP-added cheeses in terms of color, firmness, and granularity while the panelists did not detect differences ($p > 0.05$) among DP-fortified cheese. Color, firmness and granularity were scored lower in DP-added cheeses than in control, although in all cases this score was above 5 points. The differences in color and firmness noted by panelists are consistent with the instrumental results (Tables 5 and 6, respectively). The presence of DP particles in fortified cheeses was not positively evaluated by the panelist, which lowered their scores. Additionally, the incorporation of DP did not significantly ($p > 0.05$) affect the aroma, flavor, sweetness (although fructose and lactic acid content was higher in fortified cheeses), salty and fracturability of the evaluated cheeses. It is important to note that the presence of DP (at both concentrations, 4 and 8 %) in the cheeses did not negatively affect the perception of sweetness, obtaining similar values to those obtained for the control cheeses. The sensory results for fracturability align with the results for cohesiveness in the instrumental texture assessment (Table 5). Control cheeses were scored higher ($p < 0.05$) than DP-cheeses for overall acceptability, without significant differences between both concentrations of DP added. However, all scores remained above 5 points, which indicates that the evaluators generally approved all the fresh goat cheeses.

Bearing in mind that the incorporation of dates or their derivatives is not a common practice in the dairy industry, the development of studies that demonstrate the technological feasibility of their application in this matrix would allow the supply of enriched dairy products to be expanded, boosting this sector and increasing the supply of dairy products for the consumer. Furthermore, the fact that the co-products of date industrialisation can be used as a new food ingredient (date paste) that is stable and available all year round, could provide great added value to date production, boosting local development and creating jobs. From the point of view of scientific-technological feasibility, it has been proven that the incorporation of date paste at concentrations of up to 8 % does not interfere in the production process of fresh goat's cheese, i.e. it would not require the incorporation of additional processes or machinery to the traditional ones. However, further research would be needed to scale it up to an industrial level, optimising both the processing conditions and the concentrations of date paste required.

4. Conclusions

The use of date coproducts (such as date paste) for the fortification of fresh goat cheese is a promising sustainable approach, that is both technologically feasible and effective to valorize date production and industrialization. This approach contributes to the reduction of agro-industry waste while, increasing the valued of the resulting cheeses, aligning with sustainable food production practices and the circular economy principles. Date paste properly enhances cheeses by improving their fatty acid profile, increasing beneficial bacteria counts and incorporating micronutrients into the casein matrix, all while preserving the cheese's technological qualities without minimal impact on its sensory attributes.

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Ethical statement

Protocols for sensory analysis were approved (ref. PRL.DTA.JPA.05.21) by the Project Evaluation Office of the Miguel Hernández University (OEP, UMH, Elche, Alicante, Spain). Participants gave informed consent via the statement "I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for sensory evaluation.

CRediT authorship contribution statement

Clara Muñoz-Bas: Writing – original draft, Methodology, Investigation, Formal analysis. **Nuria Muñoz-Tebar:** Writing – original draft, Methodology, Formal analysis, Data curation. **Manuel Viuda-Martos:** Visualization, Methodology, Conceptualization. **Estrella Sayas-Barberá:** Resources, Investigation, Funding acquisition. **José Angel Pérez-Alvarez:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Juana Fernández-López:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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7.7. PUBLICACIÓN 7

Quality properties of innovative goat milk kefir enriched with date paste (*Phoenix dactylifera* L.) and whey derived from goat cheese production

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Quality Properties of Innovative Goat Milk Kefir Enriched with Date Paste (*Phoenix dactylifera* L.) and Whey Derived from Goat Cheese Production

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Abstract: The aim of this work was to evaluate the impact of fortifying goat milk kefir with high-value ingredients (3% and 6% date paste, and 25% and 50% goat milk substitution with date–cheese whey), derived from the valorization of date coproducts, on its nutritional (proximate composition and mineral profile), technological (pH, acidity, viscosity, color, sugar and organic acid content), microbiological and sensory properties. Both ingredients enhanced the growth and stability of the kefir starter culture, thereby improving the probiotic potential of date-added kefir and also its nutritious quality (lower fat content and higher protein content). The mineral profile of kefir was improved only when the date paste was added. Date paste could be used as an ingredient in fortified kefir (up to 6%) without altering its flow properties because it was perfectly integrated within the milk matrix. The use of date–cheese whey as a goat milk substitution (>25%) decreased the typical kefir viscosity, inducing an excessive phase separation negatively valued by consumers. Consumers preferred the kefir with 6% date paste mainly due to its higher scores for aroma, flavor, sweetness and acidity.

Keywords: kefir; goat; date; coproducts; fortification

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1. Introduction

Dairy products are common and essential foods in the human diet due to the unique properties and components of milk (i.e., calcium content, protein and vitamins D and B12, among others) [1,2]. These nutrients are crucial for maintaining strong bones, supporting muscle health and promoting overall well-being [3,4]. Moreover, dairy can be a source of probiotics, which contribute to gut health and enhance digestion [4]. Additionally, the versatility of milk has enabled the production of a wide range of milk-based foods (fresh, aged, fermented, flavored, etc.) tailored to meet cultural preferences, regional availability and consumer demands. The dairy industry has leveraged this versatility to lead the way in developing new dairy products by the addition of health-promoting ingredients such as vitamins, dietary fiber, omega-3 fatty acids or tocopherols. This has resulted in functional and/or fortified dairy products that are highly accepted by current consumers [5–7].

In this sense, kefir is a self-carbonated alcoholic fermented milk, originating from the Caucasian Mountains, that can be made from any type of milk (cow, goat or sheep milk) [8,9]. The selection of goat milk versus cow milk for kefir production is mainly based

on the better nutritional profile, digestibility and technological suitability [10,11]. Goat milk has higher levels of calcium, potassium and vitamin A compared to cow milk. Its fat globules are smaller and more easily digestible, making it a suitable alternative for individuals with lactose intolerance or a cow milk protein allergy [12]. The presence of medium-chain fatty acids in goat milk contributes to its potential health benefits, such as improved metabolism and enhanced immune function [10]. Additionally, goat milk exhibits excellent technological aptitude while maintaining its nutritional integrity and flavor profile.

The fermentation process of kefir involves the symbiotic growth of various microorganisms, including lactic acid bacteria (*Lactobacillus* spp., *Streptococcus* spp., *Lactococcus* spp. and *Leuconostoc* spp.), yeasts (*Saccharomyces* spp., *Candida* spp., *Torula* spp. and *Kluyveromyces* spp.) and acetic bacteria, which can help to restore the gut microbiota balance, thereby enhancing digestive health, immune function and overall well-being [13]. In the large intestine, probiotic bacteria play a key role in metabolizing dietary carbohydrates that would otherwise be indigestible (e.g., prebiotics), having a favorable influence on the metabolism of proteins and ammonia in the colon [14]. Likewise, lactic acid bacteria help absorb lactose and have a beneficial effect on many digestive system diseases [4].

Kefir is a refreshing fermented dairy product known for its smooth, flowing consistency, uniform and bright appearance and mild, yeast-like taste and flavor [15]. Additionally, it is a rich source of sugars, organic acids, alcohols and esters which are produced during fermentation [16]. Most kefir consumers choose it for its health benefits, while the main reason for non-consumption is a dislike of its taste [17]. Beyond probiotic microorganisms, there is a growing consumer interest in diets enriched with fruits and vegetables to improve both the functional and sensory qualities of food [18,19]. Also, consumers are increasingly concerned about the sustainability of the food production chain [20,21]. In this context, recent efforts have been made to valorize the coproducts from the agri-food industry, given their richness in valuable compounds such as dietary fiber, vitamins, sugars and polyphenolic compounds. This approach helps reduce waste production while contributing to the UN's Sustainable Development Goal 12: Responsible Consumption and Production [22–24].

In this regard, coproducts from the production and commercialization of fresh dates (*Phoenix dactylifera* L.) have been processed by applying non-pollutant basic operations (e.g., grinding, soaking and drying) to obtain intermediate and stable high-value ingredients which are ready to be reinstated in the food chain [25]. The resulting ingredients (date water, date paste or date powder), which vary in appearance, physicochemical properties and composition, offer significant versatility for their incorporation in a wide range of food matrices [11,22,25]. Notably, these ingredients retain much of the nutritional value of the original raw material (fresh dates of the Confitera cv), being rich in sugars, organic acids, dietary fiber, minerals, vitamins and polyphenolic compounds [25–27]. In addition, some of them have demonstrated relevant biological properties such as a prebiotic effect [28], which is particularly significant in fermented dairy products [11,29,30].

Growing awareness on digestive health among consumers has forced manufacturers to develop new probiotic beverages. In Europe, sales of kefir are increasing because probiotic foods and drinks are popular. The global kefir market size was valued at USD 1.23 billion in 2019 and is projected to reach USD 2.40 billion by 2032. New product developments focusing on emerging trends such as organic, lactose-free, flavored beverages and with sustainable ingredients and process are expected to drive the growth of the kefir industry in the coming years. This last aspect (sustainability) is where our work is mainly focused, because the valorization of coproducts from the agri-food industry would contribute to

reaching zero waste and to the circular economy. In any case, this fact will not be negative for the industry or for consumers.

The aim of this work was to evaluate the impact of fortifying goat milk kefir with high-value ingredients derived from date coproducts on its nutritional composition and physicochemical, microbiological and sensory properties.

2. Materials and Methods

2.1. Materials

The goat milk was collected from the farm of the Miguel Hernández University (Orihuela, Alicante, Spain) during the spring of 2024 and was kept refrigerated (4 °C) until its use less than 12 h later (protein: 3.2 g/100 g, fat: 4.6 g/100 g and carbohydrates: 4.3 g/100 g). Date paste (protein: 1.2 g/100 g, fat: 0.4 g/100 g and carbohydrates: 49.7 g/100 g) was obtained, as an intermediate ingredient, from the coproducts from fresh dates (Confitera cv.) from Elche (Alicante, Spain), following the process described by Muñoz-Bas et al. [25]. The whey (protein: 0.5 g/100 g, fat: 0.3 g/100 g and carbohydrates: 4.1 g/100 g) was obtained from the previous production of fresh goat's cheese with 8% added date paste [11]. The starter culture (Kefir Yogotherm) was purchased from Abiasa (Pontevedra, Spain) and contained the following strains: *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subps. *diacetylactis*, *Lactobacillus rhamnosus* and *Kluyveromyces marxianus*.

2.2. Kefir Making

Goat milk was pasteurized at 60 °C for 30 min in a Thermomix TM6 Vorwerk (Wuppertal, Germany) and then cooled at 25 °C. Five batches of one liter each were made: one control kefir (C), two batches with 3% and 6% date paste (DP3 and DP6) and another two batches with a 25% and 50% replacement of milk by cheese whey (WH25 and WH50). First, the milk was mixed with the date paste or cheese whey and then the kefir starter culture was added at the dosage established by the company (1 g/L of milk). Subsequently, the kefir was poured into 100 mL containers and incubated for 20–22 h at 25 °C. Finally, the samples were refrigerated overnight (less than 24 h) until further analysis.

2.3. Kefir Analysis

2.3.1. Proximate Composition

Moisture (AOAC 925.45) and ash (AOAC 923.03) were analyzed following AOAC methods [31]. On the other hand, fat, protein and totals solids were determined with a MilkoScan FT120 (FOSS, Hilleroed, Denmark) calibrated for cream. All measurements were performed in triplicate.

2.3.2. Physicochemical Properties

pH and Acidity

The pH of the kefir was measured with a pH-meter Sension + pH31 (HACH Iberia, L'Hospitalet de Llobregat, Barcelona, Spain). On the other hand, acidity was measured by titration with NaOH 0.11 N using phenolphthalein as an indicator, with the results expressed as ^{0}Dornic (^{0}D). Acidity and pH were determined in triplicate.

Color Properties

CIELAB color coordinates (L*, a* and b*) were performed in triplicate and were measured using a spectrophotocolorimeter (Konica Minolta, Osaka, Japan) with a D65 illuminant and a 10° observer angle. From the color coordinates, the psychophysical attributes, chroma (C*), hue (H*) and the whiteness index (WI) [32] were calculated:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$H^* = \text{arctg } b^*/a^* \quad (2)$$

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (3)$$

Viscosity

Viscosity was determined in triplicate with the rotational viscometer J.P. Selecta ST-2020-L (Barcelona, Spain) with the S2 spindle at 40 rpm and the results were expressed in mPa.s. [33].

2.3.3. Mineral Composition

The mineral composition was measured using the inductively coupled plasma mass spectrometry (ICP-MS) Shimadzu MS-2030 (Shimadzu, Kioto, Japan) using the conditions described by Muñoz-Bas et al. [11]. To analyze the mineral content, the samples were lyophilized (Freeze dryer Alpha 2–4, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and digested with nitric acid (67%) and hydrogen peroxide (33%) using a microwave system. The results were measured in triplicate and expressed as mg/100 g wet weight of kefir samples.

2.3.4. Organic Acids and Sugars

Organic acids and sugars were extracted using the method described by Muñoz-Bas et al. [25]. After extraction was completed, 20 µL of each sample was injected into an HPLC (Hewlett-Packard 1100 series, Woldbronn, Germany) with a Supelco SupelcogelTM c-610H column (300 mm × 7.8 mm). The elution buffer used was orto-phosphoric acid in water (0.1% *v*/*v*) and organic acids were quantified by measuring the absorbance at 210 nm with a diode array detector (DAD G-1315 A, Agilent, Santa Clara, CA, USA), while sugars were evaluated using a refractive index detector (RID G1362A, Agilent, Santa Clara, CA, USA). The peaks were identified by comparing their retention times with those of the standards (organic acids, monosaccharides and oligosaccharides from Supelco, Sigma-Aldrich, St. Louis, MO, USA) and quantified using the regression formula derived from the standards.

2.3.5. Microbiology

The microbiological quality (total aerobic count, LAB, Enterobacteriaceae and molds and yeast) of kefir with date coproducts was evaluated following the procedure described by Muñoz-Tebar et al. [34]. A total of 10 g of each kefir sample was homogenized with 90 mL of sterile peptone water 0.1% (*w*/*vol*) in a masticator for 60 s. Decimal dilutions were then made using the same medium and 0.1 mL was manually seeded, in duplicate, on MRS agar for *Lactobacillus* spp. and on M17 agar for *Streptococcus* spp. Petri dishes were incubated at 37 °C for 48 h in the case of *Streptococcus* spp., and at 37 °C for 48 h in an anaerobic chamber with an Anaerocult A (Merck, Darmstadt, Germany) for *Lactobacillus* spp. Molds and yeasts and Enterobacteriaceae were also counted using the same dilutions, and 1 mL of these dilutions was seeded, in duplicate, in Petrifilm plates (3 M, Madrid, Spain) for molds and yeasts and for Enterobacteriaceae. Petrifilm dishes were incubated at 37 °C for 24 h for the Enterobacteriaceae, and at 25 °C for 120 h for molds and yeasts. Plates with 30–300 colony-forming units (CFU) were manually counted and the results were expressed as log CFU/g of kefir.

2.3.6. Sensory Analysis

To evaluate the acceptance of kefir with date paste, a consumer study was carried out at the Polytechnic School of Orihuela of Miguel Hernández University (UMH) with fifty consumers (55% female, 45% male, aged from 20 to 65). Prior to commencing the

analyses, all participants were briefed on the distinct features of the product they would be tasting and the nature of the analysis itself. They also provided their written informed consent. This study received approval from the Responsible Research Office at Miguel Hernández University (OIR-Reg. 211128200759, Ref. PRL.DTA.JPA.05.21, UMH, Elche, Alicante, Spain). The study was carried out in a special room for sensory analysis studies which complied with international standards [35]. Participants of the study were seated in individual booths under TL 5 fluorescent lighting (Philips-Iberica, Madrid, Spain) with an intensity of approximately 350 lx. The kefir samples were served in small transparent plastic cups, each with a label with three different digits and in a random order. Consumers evaluated the following seven attributes (color, odor, flavor, sweetness, acidity, viscosity and overall acceptability). A discrete nine-point hedonic scale ranging from "dislike extremely" (1) to "like extremely" (9) was used [36].

2.4. Statistical Analysis

Statistics were measured using SPSS (IBM SPSS Statistics version 26). A one-way ANOVA (using a 95% confidence level) was performed on the results obtained to determine any significant differences between the kefir control and the samples containing date paste (3% and 6%) and cheese whey (25% and 50% milk substitution). When there was a significant difference, a Tukey test was performed to check for differences among the kefir formulations.

3. Results and Discussion

3.1. Proximate Composition

Table 1 shows the proximate composition of kefir influenced by the incorporation of date paste and goat cheese whey, where it can be seen that the values observed in all samples were within the proximate composition ranges reported in the literature for goat milk kefir [37–39]. An increase in moisture and ash content was observed in all samples (both those containing whey and date paste) compared to the control kefir, with the highest values found in the kefir made with 50% cheese whey as a milk substitute. The significant increase in moisture content in the WH50 sample may be attributed to the whey itself, as whey is primarily water (>90%; [40,41]). Similarly, fat and protein content significantly decreased ($p < 0.05$) in the samples with date paste and cheese whey compared to the control, dropping from 4.87% to 2.68% fats, and from 3.73% to 1.63% proteins in the WH50 kefir. Finally, for total solids, a significant decrease ($p < 0.05$) was observed in kefir containing date paste (DP3 and DP6, $p > 0.05$) and an even greater decrease in kefir containing whey (WH25 and WH50, $p < 0.05$). Overall, the differences found among the formulations are likely related to the proximate composition of the date paste (low protein and fat content) and cheese whey (low fat and protein content and high water content) reported in Section 2.1.

Table 1. Nutritional composition (g/100 g) of goat kefir fortified with date coproducts (mean ± sd).

Sample	Protein	Fat	Ash	Moisture	Total Solids
Control	3.73 ± 0.12 ^{a}	4.87 ± 0.04 ^{a}	0.72 ± 0.01 ^{c}	85.42 ± 0.32 ^{c}	4.85 ± 0.21 ^{a}
DP3	2.39 ± 0.01 ^{b}	3.04 ± 0.00 ^{b}	0.76 ± 0.02 ^{bc}	86.56 ± 0.83 ^{bc}	4.36 ± 0.06 ^{b}
DP6	2.46 ± 0.03 ^{b}	3.22 ± 0.80 ^{b}	0.85 ± 0.05 ^{b}	86.73 ± 0.19 ^{ab}	4.40 ± 0.07 ^{b}
WH25	2.11 ± 0.07 ^{c}	3.17 ± 0.04 ^{b}	0.82 ± 0.02 ^{bc}	86.08 ± 0.59 ^{bc}	3.81 ± 0.01 ^{c}
WH50	1.63 ± 0.01 ^{d}	2.68 ± 0.17 ^{c}	0.98 ± 0.04 ^{a}	87.96 ± 0.62 ^{a}	3.16 ± 0.05 ^{d}
p-value	0.000	0.000	0.000	0.001	0.000

$^{a–d}$ Different letters between rows mean significant differences ($p < 0.05$). DP3: kefir with 3% date paste; DP6: kefir with 6% date paste; WH25: kefir with 25% of milk substituted by date–cheese whey; WH50: kefir with 50% of milk substituted by date–cheese whey.

In comparison to similar studies, Erzhad et al. [42] also reported lower protein and higher ash values when they incorporated red fruit extracts in probiotic goat milk beverages, while Tawfeck et al. [43] observed lower fat levels in fermented goat milk drinks enriched with date palm compared to control kefir.

3.2. Mineral Profile

The mineral content of kefir formulated with date paste and cheese whey is presented in Table 2, showing significant changes in all minerals analyzed ($p < 0.05$). The minerals found in greater amounts were sodium (95.06–423.58 mg/100 g), potassium (95.45–131.37 mg/100 g), calcium (64.04–88.68 mg/100 g), phosphorus (39.92–64.61 mg/100 g) and magnesium (8.04–11.43 mg/100 g), while iron, copper, manganese and zinc were found in small amounts (<1 mg/100 g). Notably, the addition of date paste resulted in a significant increase in Ca, P and Zn levels, whereas samples containing cheese whey showed the lowest values for these minerals. Similarly, K, Mg, Cu and Mn levels were higher in kefir enriched with date paste, while the substitution of milk with cheese whey had no significant effect on them as their values were similar to the control ($p > 0.05$). The observed increases in the Ca, K and Mg content in kefir enriched with date paste are primarily attributed to the mineral content of the date paste itself, which contains substantial amounts of these macroelements (K: 359.36 mg/100 g, Ca: 272 mg/100 g, Mg: 165.05 mg/100 g) [25]. Since the most valuable mineral in kefir is calcium, the reformulation of kefir with up to 25% date paste or even with cheese whey did not cause a decrease in its content. On the other hand, it was observed that the iron content decreased when incorporating 50% date paste and cheese whey, while the values remained the same in the control kefir and the one containing 25% whey. However, these differences were practically insignificant, as Fe concentrations across all samples were consistently low (0.04–0.05 mg/100 g). The most pronounced effect was found in the sodium content, which was significantly increased in the kefir formulated with cheese whey, obtaining values almost 5 times higher (WH50) compared to the control (95.06 vs. 423.58 mg/100 g). Sodium, part of the milk's aqueous phase, is found in greater quantities in whey, also being influenced by the salt used in cheese processing which could explain the highest content of sodium being present in kefir formulated with cheese whey compared to the control or those containing date paste. In contrast, copper, iron and zinc, which are more strongly associated with caseins in ruminant milk, are present in lower concentrations in whey [44].

Table 2. Mineral profile (mg/100 g) of kefir fortified with date coproducts (mean ± sd).

Sample	Control	DP3	DP6	WH25	WH50	*p*-Value
Ca	82.09 ± 1.70 ^{b}	88.16 ± 0.86 ^{a}	88.68 ±1.29 ^{a}	80.32 ± 0.28 ^{b}	64.04 ± 1.28 ^{c}	0.000
Cu	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{ab}	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.000
Fe	0.05 ± 0.00 ^{a}	0.04 ± 0.00 ^{b}	0.04 ± 0.00 ^{b}	0.05 ± 0.00 ^{a}	0.04 ± 0.00 ^{b}	0.000
K	96.64 ± 1.84 ^{b}	131.25 ± 0.45 ^{a}	131.37 ± 0.75 ^{a}	97.74 ± 1.81 ^{b}	95.45 ± 2.48 ^{b}	0.000
Mg	8.47 ± 0.11 ^{c}	10.39 ± 0.10 ^{b}	11.43 ± 0.08 ^{a}	8.27 ± 0.45 ^{c}	8.04 ± 0.19 ^{c}	0.000
Mn	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.000
Na	95.06 ± 5.25 ^{d}	104.83 ± 1.57 ^{c}	111.23 ± 3.37 ^{c}	376.86 ± 0.84 ^{b}	423.58 ± 0.66 ^{a}	0.000
P	54.33 ± 0.01 ^{c}	58.06 ± 0.77 ^{b}	64.61 ± 0.83 ^{a}	55.25 ± 0.68 ^{c}	39.92 ± 0.63 ^{d}	0.000
Zn	0.23 ± 0.01 ^{c}	0.30 ± 0.001 ^{b}	0.36 ± 0.02 ^{a}	0.22 ± 0.01 ^{c}	0.17 ± 0.01 ^{d}	0.000

$^{a–d}$ Different letters between columns mean significant differences ($p < 0.05$). DP3: kefir with 3% date paste; DP6: kefir with 6% date paste; WH25: kefir with 25% milk substituted by date–cheese whey; WH50: kefir with 50% milk substituted by date–cheese whey.

In a similar study, Tawfek et al. [43] developed fermented goat milk beverages with date palm, reporting comparable results in mineral content with significant increases in

potassium, magnesium, iron and zinc content. So far, the available literature on minerals in fermented goat milk products has been limited since most studies have mainly focused on the mineral composition of the milk itself. Therefore, the findings of the present study are particularly valuable, offering new insights that can support the development of innovative fermented goat milk products, specifically those enriched with date paste and cheese industry by-products such as whey.

3.3. Organic Acids and Sugars

The analysis of organic acids and sugars in goat kefir (Table 3) revealed that lactic acid was the only organic acid consistently detected across all formulations. The lowest levels ($p > 0.05$) were observed in samples containing whey, primarily due to the lactic acid content typically found in cheese whey, as noted by Borba et al. [45]. This reduced lactic acid content in whey results from the cheese-making process, which includes milk pasteurization and the addition of rennet to coagulate casein and form curds, without significant lactobacilli activity to convert lactose into lactic acid [45].

Table 3. Sugar and organic acid contents (mg/g) of kefir fortified with date coproducts (mean ± sd).

Sample	Lactic Acid	Fructose	Lactose
Control	5.55 ± 0.16 ^{b}	ND	27.06 ± 0.89 ^{c}
DP3	6.28 ± 0.07 ^{a}	4.37 ± 0.02 ^{b}	27.25 ± 0.72 ^{c}
DP6	6.42 ± 0.09 ^{a}	5.68 ± 0.02 ^{a}	26.93 ± 0.19 ^{c}
WH25	3.21 ± 0.18 ^{c}	ND	34.22 ± 0.04 ^{b}
WH50	2.89 ± 0.17 ^{d}	ND	36.38 ± 0.03 ^{a}
p-value	0.001	0.000	0.000

$^{a–d}$ Different letters between rows mean significant differences ($p < 0.05$). DP3: kefir with 3% date paste; DP6: kefir with 6% date paste; WH25: kefir with 25% milk substituted by date–cheese whey; WH50: kefir with 50% milk substituted by date–cheese whey.

In formulations enriched with date paste, an increase in lactic acid was noted compared to the control (from 5.55 to 6.42 mg/g). This increase in lactic acid content was mainly due to the fact that LAB such as *Lactobacillus* spp. use glucose as a substrate during fermentation to produce lactic acid [46].

Regarding sugars, lactose was the predominant sugar found in all goat kefir formulations, while fructose was only detected in samples containing date paste. This was because fructose is one of the primary sugars in date paste, as previously reported (137.72 mg/g) [25]. On the other hand, the highest lactose levels were observed in samples enriched with cheese whey, primarily due to the naturally high lactose content in whey [44,45]. However, the addition of date paste did not affect the lactose content of kefir, with values remaining comparable to the control.

3.4. Physicochemical Properties

Considering that kefir is defined as "a refreshing fermented dairy product with flowing consistency, and uniform and bright appearance" [47], it seems logical that the most influential properties on its quality were those relating to the lactic fermentation process (acidity and pH), flow properties (viscosity) and color properties (L*, a* and b* coordinates and C* and H*). Table 4 shows the results of the physicochemical properties of kefir samples, demonstrating that all of them were significantly affected by the reformulation of kefir.

Titratable acidity (TA) and pH play a key role in the texture and flavor of fermented dairy products like kefir. In particular, the acidity levels in kefir are important, as both insufficient and excessive acidity can mask its characteristic buttery flavor and alter the product's structure [48]. Although there is no direct correlation between these two param-

eters, a general trend exists since pH decreases as TA increases [49]. Furthermore, both parameters are indicators of good quality, not only during the production of fermented dairy products but also of the final product. The pH and TA values (^{0}Dornic) of the different kefir samples are shown in Table 4, showing that the addition of DP slightly decreased the pH values of kefir in a concentration-dependent manner ($p < 0.05$). Similar behavior was observed as a function of the cheese whey concentration, reaching pH values similar to those of the DP-added kefir. The decrease in pH values in kefir due to the addition of fruit extracts (such as pomegranate peel, mango peel, red prickly pear, lemon fiber and apple fiber, among others) has been previously reported [16,50,51]. This reduction was attributed to the phytochemicals and organic acids present in fruits, which can contribute to the acidic pH in kefir. In addition, fruit extracts can serve as a source of natural sugars and polysaccharides, which can be converted into glucose and eventually transformed into lactic acid by microorganisms [51]. Nevertheless, the pH values of all kefir samples were in the pH range normally reported for kefir samples (3.5–4.5; [52,53]). Titratable acidity showed a different trend, though. DP-added kefir (DP3 and DP6) showed similar TA values ($p > 0.05$) compared to the control kefir. However, the addition of whey led to a concentration-dependent decrease in TA values ($p < 0.05$), with the largest difference observed between the control and WCH50. In any case, as the values of TA normally reported for kefir samples vary between 0.50 and 1.50 g/100 mL [52,53], it could be said that all kefir samples showed values in this range. It is also important to note that none of the samples had pH values below 4.0, as such low pH levels are considered a detrimental level for probiotics [16].

Table 4. Physicochemical properties of kefir fortified with date coproducts (mean ± sd).

Sample	Control	DP3	DP6	WH25	WH50	*p*-Value
pH	4.32 ± 0.02 ^{a}	4.24 ± 0.01 ^{b}	4.21 ± 0.01 ^{c}	4.26 ± 0.01 ^{b}	4.20 ± 0.01 ^{c}	0.000
Acidicity (^{0}D)	94.00 ± 2.83 ^{a}	101.00 ± 1.41 ^{a}	96.50 ± 2.12 ^{a}	64.00 ± 1.41 ^{b}	66.00 ± 1.41 ^{c}	0.000
Viscosity (mPa.s)	1125.80 ± 60.37 ^{a}	1193.10 ± 1.18 ^{a}	1141.10 ± 6.20 ^{a}	451.60 ± 12.82 ^{b}	113.27 ± 4.02 ^{c}	0.000
L* (D65)	81.186 ± 0.74 ^{a}	73.83 ± 0.71 ^{b}	77.36 ± 0.57 ^{b}	78.48 ± 0.57 ^{c}	61.12 ± 0.39 ^{d}	0.000
a* (D65)	−0.74 ± 0.09 ^{c}	0.77 ± 0.18 ^{a}	0.67 ± 0.08 ^{a}	−0.23 ± 0.03 ^{b}	−0.62 ± 0.05 ^{c}	0.000
b* (D65)	5.14 ± 0.18 ^{b}	8.67 ± 0.54 ^{a}	9.18 ± 0.53 ^{a}	8.39 ± 0.19 ^{a}	4.55 ± 0.02 ^{b}	0.000
C* (D65)	5.19 ± 0.17 ^{b}	8.70 ± 0.54 ^{a}	9.21 ± 0.53 ^{a}	8.39 ± 0.19 ^{a}	4.59 ± 0.03 ^{b}	0.000
H* (D65)	98.24 ± 1.16 ^{a}	84.92 ± 1.14 ^{c}	85.81 ± 0.19 ^{c}	91.59 ± 0.14 ^{b}	97.73 ± 0.60 ^{a}	0.000
WI	80.48 ± 0.72 ^{a}	75.73 ± 0.41 ^{b}	76.60 ± 0.72 ^{b}	72.51 ± 0.62 ^{c}	60.84 ± 0.38 ^{d}	<0.001

$^{a–d}$ Different letters between columns mean significant differences ($p < 0.05$). WI (whiteness index); DP3: kefir with 3% date paste; DP6: kefir with 6% date paste; WH25: kefir with 25% milk substituted by date–cheese whey; WH50: kefir with 50% milk substituted by date–cheese whey.

The consistency (viscosity) of kefir is influenced by several factors, with the type of milk and the fermentation process (grain vs. lyophilized culture, and incubation temperatures) being the most important. Studies have shown that kefir made from goat and sheep milk has considerably lower viscosity compared to that made from cow milk, and that the use of kefir grains at high temperatures results in higher viscosity values [47,54,55]. The addition of DP did not modify ($p > 0.05$) the viscosity in the kefir samples at any of the added concentrations (3% and 6%). In other words, DP could be used as an ingredient in fortified kefir (up to 6%) without altering its flow properties. It has been reported that the addition of DP in other dairy products (yogurt and cheese) did not cause significant changes in their texture because DP was perfectly integrated within the milk matrix [25,34]. However, the substitution of goat milk by cheese whey significantly reduced the viscosity values, with this decrease being higher at higher substitution percentages ($p < 0.05$). These results were expected, knowing that cheese whey has a lower viscosity than goat milk.

Color plays a relevant role in the production and marketing of foods, particularly in fermented dairy products, which are typically known for their characteristic white color. It can significantly influence consumer acceptance and purchase decisions. In kefir, the color can vary due to several factors, highlighting the type of milk, the specific fermentation process and any added ingredients or additives (flavorings, colorants, etc.). The manner and extent of these color variations could even hinder the development and market launch of a new dairy product. All the reformulated kefir samples showed L* values lower than the control kefir ($p < 0.05$), with the WH50 showing the lowest L*. The lightness value in the control kefir was in line with the L* values of commercial kefir [38]. The decrease in L* values in kefir due to the addition of fruit extracts (lemon fiber, apple fiber and red prickly pear) has also been reported by other authors [16,56]. Regarding the a* and b* values, a significant increase ($p < 0.05$) was observed in DP-added kefir samples (DP3 and DP6), without any differences between the concentrations compared to the control kefir. These samples displayed the highest a* and b* values. The addition of whey only increased a* and b* values when it was added at 25% ($p < 0.05$), with WH50 showing similar a* and b* values to control kefir. The changes in redness and yellowness in kefir due to DP addition was related to the corresponding values of the date paste (a*: 2.5 and b*: 4.9; [25]) due to its orange–yellow–red pigment content (carotenes and anthocyanins) and also due to some browning compounds generated during Maillard reactions [25,57]. The color saturation behavior in kefir due to its reformulation followed the same pattern as the b* coordinate. The addition of DP increased C* values, while the use of cheese whey at 50% resulted in saturation values similar to those of the control. Hue tone decreased in reformulated kefir (except in WH50 which showed a hue similar to control; $p < 0.05$). Hue is the dimension that most people associate with an object's color; therefore, the reformulation of kefir caused a shift in hue from a lemon-yellow hue (control) to yellow–orangish (DP3 and DP6) and lemonish-yellow (WH25) [38,58]. The whiteness index decreased ($p < 0.05$) with the addition of date coproducts. In DP-added kefir, the WI did not show differences between the concentrations of DP added ($p > 0.05$). Kefir with cheese whey at the highest concentration (WH50) showed the lowest WI ($p < 0.05$). However, the WI values obtained for all reformulated kefir samples were in the range of the values reported for commercial kefir samples [38].

3.5. Microbiology

The microbiological analysis results for goat milk kefir enriched with date paste and cheese whey added with date are presented in Table 5. These results prove that the incorporation of both date paste and cheese whey did not impair the growth of kefir starter LAB and the values met the recommended minimum of 10^6 CFU/mL for live probiotic bacteria in probiotic food products [59]. The presence of Enterobacteriaceae was not detected in any kefir formulation, which confirmed that the process was carried out under optimal hygienic conditions and that the raw materials used (milk, date paste and cheese whey) were free from contamination by this type of bacteria.

For the total mesophilic aerobic counts, kefir samples enriched with date paste exhibited the highest values ($p < 0.05$) compared to the control, followed by samples containing cheese whey. In contrast, for Streptococcus sp., the control kefir showed the highest counts (8.27 log CFU/mL), followed by kefir enriched with date paste (8.23 and 8.25 log CFU/mL). The lowest counts were observed in samples where part of the milk (25% and 50%) was replaced by cheese whey (8.21 and 8.18 log CFU/mL, respectively). Regarding Lactobacillus sp. counts, a significant increase ($p < 0.05$) was observed with the incorporation of date paste and cheese whey (enriched with 8% date paste), showing values ranging from 8.21 to 8.47 log CFU/mL. This significant increase in date-enriched formulations suggests

that their probiotic potential could be related to the previous prebiotic activity of date coproducts, as previously demonstrated in the study carried out by Muñoz-Bas et al. [28]. Additionally, the improvement of microbial growth in samples fortified with date paste might be linked to its mineral and phenolic compound contents, which could serve as prebiotic substrates in functional fermented products, as well as its carbohydrate content, which may stimulate the LAB [60]. However, the interaction between bacteria and phenolic compounds is complex and can be influenced by factors such as the type and concentration of the substrate and the specific bacterial strain involved [61,62]. Finally, molds and yeasts were only detected in the samples formulated with cheese whey, but their growth was so low (<3 log cfu/mL) that they did not represent any risk for consumption. Considering that the yeast *Kluyveromyces marxianus* was in the starter used in the kefir elaboration, its absence in the kefir samples could be due to multiple factors such as competition with other microorganisms, changes in nutrient availability or the use of the culture medium for molds and yeasts instead of a selective one for yeasts.

Table 5. Microbiological counts (log CFU/ g) of kefir fortified with date coproducts (mean ± sd).

Sample	Total Aerobic Count	*Lactobacillus* sp.	*Streptococcus* sp.	Enterobacteriaceae	Molds	Yeasts
Control	8.36 ± 0.03 ^{bc}	8.21 ± 0.01 ^{d}	8.27 ± 0.01 ^{a}	ND	ND	ND
DP3	8.41 ± 0.01 ^{ab}	8.47 ± 0.01 ^{a}	8.25 ± 0.01 ^{ab}	ND	ND	ND
DP6	8.45 ± 0.00 ^{a}	8.44 ± 0.01 ^{a}	8.23 ± 0.02 ^{ab}	ND	ND	ND
WH25	8.29 ± 0.02 ^{c}	8.32 ± 0.01 ^{c}	8.21 ± 0.01 ^{bc}	ND	1.54 ± 0.09 ^{b}	1.98 ± 0.03 ^{b}
WH50	8.33 ± 0.02 ^{bc}	8.37 ± 0.01 ^{b}	8.18 ± 0.00 ^{c}	ND	1.81 ± 0.05 ^{a}	2.83 ± 0.02 ^{a}
p-value	0.003	0.000	0.004	ND	0.000	0.000

$^{a–d}$ Different letters between rows mean significant differences ($p < 0.05$). ND: not detected. DP3: kefir with 3% date paste; DP6: kefir with 6% date paste; WH25: kefir with 25% milk substituted by date–cheese whey; WH50: kefir with 50% milk substituted by date–cheese whey.

The results obtained in the present work are in line with the study conducted by Tawfek et al. [43] on date-enriched fermented goat milk beverages, which also showed higher counts of *Lactobacillus* spp. and *Sreptococcus* spp. compared to the control sample.

3.6. Sensory Analysis

The kefir samples containing the cheese whey as a milk substitute were excluded from the sensory analysis. The whey caused phase separation in the kefir, resulting in the loss of its typical appearance with the result being unsuitable for sensory analysis. The consumer panelists evaluated six attributes in each of the samples (Figure 1).

Significant differences ($p < 0.05$) were only found in acidity. The control sample had the lowest acidity score while the highest score was given to the kefir formulated with 6% DP. No significant differences were found for the rest of the attributes (color, odor, flavor, sweetness and viscosity). However, the kefir with 6% DP received the highest scores in several of these attributes, including odor, flavor, sweetness and acidity. Finally, in terms of overall acceptability, the sample that consumers liked the most was the one formulated with 6% date paste.

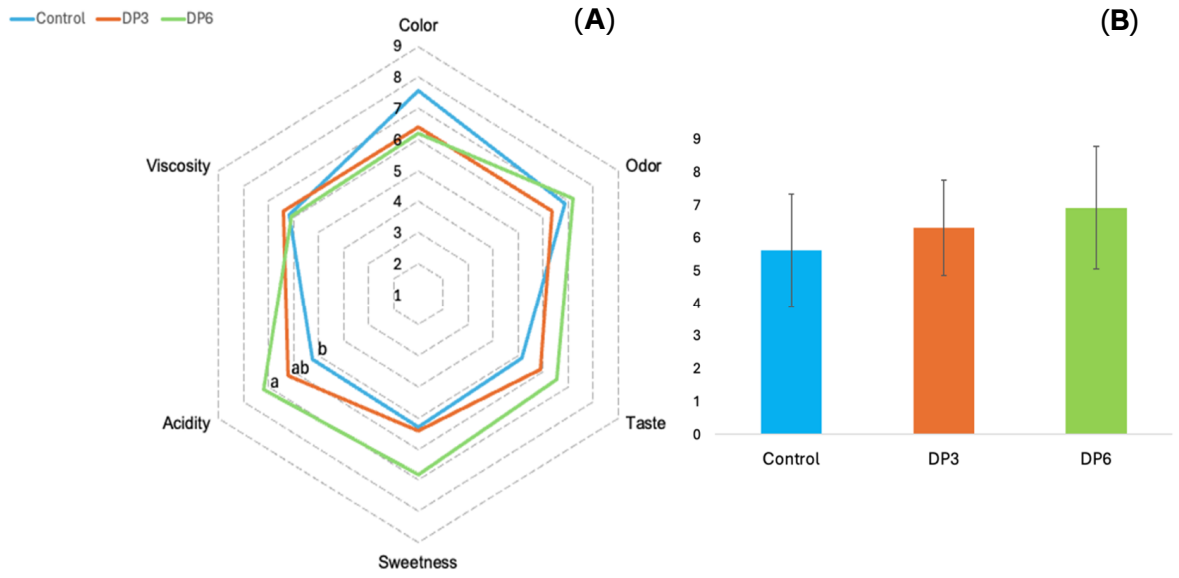


Figure 1. Sensory evaluation (**A**) and overall acceptance (**B**) of fortified kefir. Control: kefir without date paste added; DP3: kefir with 3% date paste added; DP6: kefir with 6% date paste added. Data are presented as mean ± SD. Different letters indicate statistically significant differences as determined by Tukey's HSD post hoc test ($p < 0.05$).

4. Conclusions

Fermented dairy products play an important role in supporting a healthy lifestyle and can offer numerous health advantages. Current trends emphasize the use of probiotics and plant extracts, mainly from agro-industry coproducts, to improve their sustainability. This study demonstrates that the use of date coproducts (date paste and cheese whey from the elaboration of fresh cheese with date paste) for the fortification of goat milk kefir is technologically feasible (although the use of whey would need further technological improvement), contributing to the growth of the dairy industry and expanding the range of available options in the market. The addition of both ingredients promoted the growth of probiotic microorganisms. The addition of date paste also improved the mineral profile of goat milk kefir (with the exception of sodium content). Date paste could be used as an ingredient in fortified kefir (up to 6%) without altering its flow properties, because it could be perfectly integrated within the milk matrix. The use of date–cheese whey as a goat milk substitution (>25%) decreased the typical kefir viscosity, inducing an excessive phase separation negatively valued by consumers. Consumers preferred the kefir with 6% date paste, mainly due to its higher scores for odor, flavor, sweetness and acidity. For the use of cheese whey, it should be necessary to change its processing to avoid the phase separation which was responsible for the loss of the typical kefir appearance and texture, making it less acceptable to consumers.

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Informed Consent Statement: Prior to commencing the analyses, all participants were briefed on the distinct features of the product they would be tasting and the nature of the analysis itself and provided their written informed consent.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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7.8. PUBLICACIÓN 8

Date palm (Phoenix dactylifera) and enriched fresh goat cheese: (poly)phenol profile and stability after INFOGEST 2.0 in vitro digestion method

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ABSTRACT

Date palm fruit (*Phoenix dactylifera*) is a nutrient-dense food rich in (poly)phenols. This study evaluated the profile and stability of free and bound (poly)phenols in underutilized Confitera fresh dates and in fresh goat cheese fortified with 4 % and 8 % date paste, including their behavior during *in vitro* gastrointestinal digestion. A total of 45 (poly)phenols were identified in date paste, mainly hydroxycinnamic acids and flavonols. In fortified cheeses, 22 (poly)phenols were quantified, with flavonols showing higher retention (100 %) than hydroxycinnamic acids (54.0 %). *In vitro* digestion revealed high stability and increased bioaccessibility of several date-paste (poly)phenols, such as caffeoylshikimic acid (250 %) and chrysoeriol glycoside (160 %). In contrast, the dairy matrix markedly reduced the stability of soluble-free (poly)phenols in enriched cheese, where only four compounds remained detectable, with bioaccessibility values ranging 11–43 %. The insoluble-bound fraction retained most compounds, and new compounds appeared after digestion. The highest colon-available index in enriched cheese was observed for ferulic acid (1000 %). Overall, the study indicated that the food matrix plays a decisive role in modulating the release and stability, of (poly)phenols during digestion.

1. Introduction

Date palm fruit (*Phoenix dactylifera*) is a staple food in many regions of the world, especially in North Africa. It is considered a nutrient-dense food due to its richness in carbohydrates, including simple sugars (fructose, glucose, and sucrose), as well as dietary fiber, vitamins, and minerals (Muñoz-Bas et al., 2023). Interestingly, despite their high glucose content, dates have a relatively low glycemic index (Alfaro-Viquez et al., 2018). Consequently, their use as a natural sweetener and as an ingredient in food reformulation, such as dairy and pastry products, has increased substantially in recent years (Elkot et al., 2025; Pal et al., 2024; Ranasinghe et al., 2025; Sirisena et al., 2018). Dates are a valuable source of (poly)phenols, including flavonoid glycosides, and hydroxycinnamates, among others (AlFaris et al., 2021; Echegaray et al., 2020), which have been correlated with their antioxidant activity (AlFaris et al., 2021; Fernández-López et al., 2022).

Within plant cells, (poly)phenols occur in both soluble and insoluble forms. The soluble fraction is mainly localised in the vacuole, where (poly)phenols can exist in free form or conjugated with other molecules. In contrast, the insoluble fraction is associated with the cell wall, where (poly)phenols play a structural role by being covalently bound to components such as pectin, cellulose, hemicellulose, or proteins (Acosta-Estrada et al., 2014; Shahidi & Yeo, 2016; Yao et al., 2021) These compounds represent large proportion (20 %–60 % in vegetables, fruits, and legumes/seeds) compared to soluble forms (Acosta-Estrada et al., 2014; Shahidi & Yeo, 2016), however limited information about them are available due to the time-consumption extraction method compared to soluble-free (poly)phenols extraction.

The production and commercialization of fresh dates in the southeast of Spain (Elche, Alicante) have emerged as a promising economic activity. Due to climate change, date palm cultivation is finding exceptional climatic conditions in this region, contributing to local economic development (Fernández-López et al., 2022). Moreover, several processes have been developed to valorize date co-products (dates discarded from the fresh market due to size, color, or minor damage caused by insects or handling), leading to value-added products such as date

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paste, date powder, and date juice (Muñoz-Bas, Muñoz-Tebar, Viuda-Martos, et al., 2024). These ingredients have been applied as functional components in the development of fortified or enriched foods, including dairy products (Muñoz-Bas et al., 2024a; Muñoz-Tebar et al., 2024c) and meat products (Sánchez-Zapata et al., 2011). However, beyond product innovation, it is essential to investigate their behaviour during gastrointestinal digestion to assess the stability of their main phytochemical compounds such as (poly)phenols within different food matrices.

Both date seeds and date fruits have previously been subjected to *in vitro* digestion to evaluate the recovery and stability of their (poly)phenolic profile. Djaoudene et al. (2021) assessed lyophilized samples, while Hilary et al. (2020) also investigated the stability of seed date (poly)phenols incorporated into bread. In addition, the stability of (poly)phenols from various sources, such as pomegranate juice, cinnamon powder, microencapsulated grape pomace, and coffee, has been examined after digestion in dairy matrices, mainly yogurt. These studies have highlighted strong interactions between milk proteins and (poly)phenols, which in many cases contribute to the low recovery of (poly)phenols following digestion, reaching values below 40 % in coffee-fortified yogurt. However, to the best of our knowledge, information regarding the stability of soluble-free and insoluble-bound (poly)phenols in fresh dates, as well as their behaviour in fresh cheese, remains limited. Moreover, there is limited information available on studies that characterize the (poly)phenols profile in hybrid dairy products.

Therefore, this study aimed to quantify the soluble-free and insoluble-bound (poly)phenols in fresh date palm paste (Confitera vr.) (*Phoenix dactylifera*) and to investigate their recovery in fresh goat cheese. Furthermore, the bioaccessibility and colon-available fraction of free and bound (poly)phenols in both the date paste and the fortified fresh goat cheese.

2. Materials and methods

2.1. Reagents

Green tea catechin mix (G-016), pancreatin from porcine pancreas (P7545), pepsin from porcine gastric mucosa (P6887), and bile from bovine and ovine (B8381) were purchased from Merck (Darmstadt, Germany). Six monoglycosides mixture (pelargonidin 3-glucoside, cyanidin 3-glucoside, peonidin 3-glucoside, delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside), malvidin, and malvidin 3,5-diglucoside were purchased from Biolink Group-Polyphenols AS (Sandnes, Norway). The following individual (poly)phenol standard: 4-hydroxybenzoic acid, apigenin, apigenin 7-*O*-glucoside, caffeic acid, catechin, catechin-3-gallate, cinnamic acid, crypto chlorogenic acid, diosmetin-7-*O*-rutinoside (diosmin), diosmetin-7-*O*-neohesperidoside (neodiosmin) ellagic acid, epicatechin, eriodictyol-7-*O*-rutinoside (eriocitrin), ferulic acid, gallic acid, gallocatechin gallate, hesperetin 7-rhamnoglucoside (hesperidin), isorhamnetin-3-*O*-glucoside, kaempferol, luteolin, myricetin, naringenin 7-O-neohesperidoside (naringin), naringenin 7-*O*-rutinoside (narirutin), *p*-coumaric acid, isosakuranetin-7-*O*-neohesperidoside (poncirin), protocatechuic acid, quercetin, quercetin 3-β-d-glucoside, quercetin 3-*O*-rhamnoside (quercitrin), quercetin 3-*O*-rutinoside (rutin), rosmarinic acid, sinapic acid, syringic acid, vanillic acid, and vanillin, were purchased from Merck (Darmstadt, Germany) or EXTRASYNTHESE SAS (Z.I Lyon Nord, France). All other reagents were purchased from PanReac ApliChem (Barcelona, Spain).

2.2. Materials

Date paste (DP) was obtained from coproducts of fresh dates (*Phoenix dactylifera*, Confitera cv.) on the Tamar ripening stage followed the process described by Muñoz-Bas et al. (2024c). In brief, discarded date palm from Elche palm grove (Alicante, Spain) were pitted and minced. The main components of the date paste were moisture, sugars, and total dietary fiber, with values ranging from 48.0 to 48.5 g/100g, 31.0–31.5 g/100g, and 18.3–18.9 g/100g, respectively.

The obtained date paste was employed to enriched to 4 % and 8 % goat fresh cheese following the procedure previously described by Muñoz-Bas et al. (2024b). The three fresh cheese developed, control cheese (without date palm addition), fresh goat cheese with 4 % addition of date palm paste (DPC-4) and fresh goat cheese with 8 % addition of date palm paste (DPC-8) were made using goat milk that was collected from the farm of the Miguel Hernandez University (Orihuela, Alicante, Spain) and was pasteurized (72 °C/15 s) before using it.

2.3. (Poly)phenol extraction

(Poly)phenol extraction was performed in four different matrices: date palm paste (DP), fresh goat cheese control (CT), fresh goat cheese with 4 % addition of date palm paste (DPC-4) and fresh goat cheese with 8 % addition of date palm paste (DPC-8). Two complementary extraction methods were used to recover free-soluble (poly)phenols and the insoluble-bound compounds.

2.3.1. Soluble-free (poly)phenols

For the extraction of free (poly)phenols, the procedure described by Lucas-González et al. (2023) was followed with minor modifications. Briefly, 5 g of each sample was weighed and mixed with 50 mL methanol-water solution (80:20, v/v). The mixture was homogenized for 1 min using a T-25 Digital Ultraturrax (IKA Works, Spain) and subsequently centrifuged at 7000 g for 10 min at 4 °C. The supernatant was collected in a 250 mL flask. The extraction was repeated using 50 mL of an acetone-water solution (70:30, v/v). Both supernatants were combined and concentrated under reduced pressure using a rotary evaporation (Rotavapor®, Büchi, model R-200, Switzerland). The dry residue was reconstituted in 10 mL of distilled water, passed through a C18 cartridge (CHROMAFIX®) and eluted with formic acid:methanol for HPLC analysis (1:99, v/v).

2.3.2. Insoluble-bound (poly)phenols

For the extraction of insoluble-bound (poly)phenols, the pellet obtained after the extraction of free (poly)phenols was used, following the methodology described by (Mpofu et al., 2006) with the modification reported by Lucas-González et al. (2023). Briefly, the pellet was suspended in 40 mL of NaOH (4 mol/L) and stirred for 4 h in the dark using a rotary agitator (Intelli-Mixer RM-2M, ELMI, Latvia). Then, the pH was adjusted to 2.0 with HCl (6 mol/L) and the mixture was centrifuged at 10 000 g for 20 min at 4 °C. The resulting supernatant was transferred to separating funnel, mixed with 30 mL of ethyl acetate, shaken for 2 min and left to stand for 24 h. The aqueous phase was washed twice with 20 mL of ethyl acetate. The organic phase was filtered and subsequently evaporated in a rotary evaporator (Rotavapor®, Büchi, model R-200, Switzerland). Finally, the residue was reconstituted in 10 mL of water, purified through C18 solid-phase extraction cartridge (CHROMAFIX®), and eluted with formic acid: methanol for HPLC analysis (1:99, v/v).

2.4. Detection and identification of (poly)phenols

(Poly)phenol analysis was performed by High-Performance Liquid Chromatography (HPLC; Hewlett-Packard Series 1200) using a C18 column (Mediterranean Sea18, 25 × 0.4 cm, 5 μm particle size; Teknokroma, Barcelona, Spain). The mobile phases consisted of acetonitrile and formic acid:water (1:99, v/v), applied under gradient conditions as described by Genskowsky et al. (2016). Detection was monitored at 280, 325, 360, and 520 nm. Compounds were tentatively identified by comparing retention times and UV–Vis spectra with authentic standards (Supplementary Fig. 1); when standards were unavailable, identification was supported by literature data (Farag et al., 2014; Hilary et al., 2020). Quantification was based on calibration curves from available standards.

2.5. In vitro gastrointestinal digestion

In vitro digestion was performed following the INFOGEST 2.0 protocol (Brodkorb et al., 2019). Prior to digestion, samples were conditioned. For the date paste, a tomato paste-like consistency was obtained by mixing 2 g of date paste with 3 g of distilled water, and this mixture was used for digestion. Cheese samples (control and date-paste-enriched) were minced using a coffee mill. Digestion began by mixing 5 g of sample with 5 mL of simulated salivary fluid without α-amylase (1:1, w/v), followed by shaking at 70 rpm, at 37 °C for 2 min. Subsequently, 10 mL of simulated gastric solution (pepsin 2000 U/mL) was added, the pH was adjusted to 3.0 and samples were incubated for 2 h in a water bath orbital (37 °C and 70 rpm). To stop the gastric phase, the pH was adjusted to 7.0, and 20 mL of simulated intestinal fluids (pancreatin 100 U/mL trypsin; bile salts 10 mM) were added and incubation continued for another 2 h. After digestion, the samples were centrifuged at 7000 g for 10 min. The resulting supernatant was used to determine soluble-free (poly)phenols; it was filtered, passed through a C18 solid phase extraction cartridge (CHROMAFIX®), and resuspended in formic acid:methanol (1:99, v/v) for HPLC analysis. The pellet was used for extraction of insoluble-bound (poly)phenols as described on section 2.3.2.

2.6. (Poly)phenol stability and bioaccessibility after in vitro gastrointestinal digestion

The stability of (poly)phenols throughout cheese manufacture was evaluated by calculating the recovery index, defined as the ratio between the theoretical (poly)phenol content predicted from the formulation and the amount experimentally determined concentration in the final product. Moreover, the stability of (poly)phenols following *in vitro* gastrointestinal digestion was assessed through the bioaccessibility index and the colon-available index, calculated according to Equations (1) and (2) (Lucas-González et al., 2021).

$$\textit{Bioaccessibility index}\ (\%) = \frac{CSF}{TP_u}\ x\ 100 \qquad \text{(eq 1)}$$

Where,

CSF: Chyme soluble fraction (soluble-free (poly)phenols after intestinal digestion) (μg/g)

TPu: Total (poly)phenol content in the undigested sample, expressed either as soluble-free alone or as the sum of soluble-free and insoluble-bound fractions, depending on the (poly)phenol (μg/g). For the calculation of the total bioaccessibility of all (poly)phenols present in the samples, the sum of both fractions (soluble-free + insoluble-bound) was used.

$$\textit{Colon availible index}\ (\%) = \frac{BPi}{BP_u}\ x\ 100 \qquad \text{(eq 2)}$$

Where,

BPi: Insoluble-bound (poly)phenols after intestinal phase (μg/g)

BPu: Insoluble-bound (poly)phenols on undigested samples (μg/g)

2.7. Statistical analysis

All analyses were performed in triplicate. Statistical treatment of the data was conducted using SPSS Statistics v.26, (IBM Corp., Armonk, NY, USA). A one-way ANOVA (95 % confidence level) was applied to assess significant differences among samples, followed by Tukey's post-hoc test to determine significant differences ($P < 0.05$).

3. Results and discussion

3.1. (Poly)phenol profile on date palm paste

A total of fifty (poly)phenols were detected in both the free and bound fractions Confitera date paste (Table 1). Of these, thirteen compounds were unequivocally confirmed by comparison with pure standards. The remaining compounds were tentatively identified by comparing their retention times and UV–Vis absorption spectra with those available standards and with data reported in the literature (Table 1), except for compounds no.10 and no. 34 (Table 1), which could not be identified. Each subfamily of (poly)phenols exhibits a characteristic absorbance profile and elutes according to decreasing polarity. Typically, phenolic acids and flavonoid diglucosides elute first, followed by monoglucosides, acylated monoglucosides, and finally free aglycones (Farag et al., 2014) (Supplementary Figs. 1 and 2).

The greatest diversity of compounds was observed in the soluble-free fraction, in which thirty compounds were detected. Hydroxycinnamic acids and their derivatives were the most abundant group (ten compounds), followed by flavonols (nine compounds), and flavanones (six compounds). In contrast, the insoluble-bound fraction was dominated by flavan-3-ols (ten compounds). Only quercetin and ferulic acid were detected in both fractions. Among the predominant soluble-free compounds, chrysoeriol glycoside 1 exhibited the highest concentration, followed by caffeic acid and caffeoylshikimic acid 1 (Table 2).

Among the hydroxycinnamic acids detected, caffeic, ferulic and chlorogenic acids were confirmed using analytical standards. The remaining compounds were tentatively identified as caffeic acid or *p*-coumaric acid conjugates. Compounds exhibiting a lower λ_{max} than the aglycones (318–320 nm, instead 324–328 nm) and eluting earlier were considered glycosylated derivatives. Previous studies have also reported a wide diversity of hydroxycinnamic acids in dates, including caffeoylshikimic acid glycosides, and glycosides of caffeic and ferulic acids in Deglet Nour and Medjool cultivars (Hammouda et al., 2013; Khallouki et al., 2018; Mansouri et al., 2005; Alfaro-Víquez et al., 2018).

Regarding flavonoids, glycosylated and methylated derivatives of quercetin and luteolin were the predominant compounds in Confitera date paste. In particular, rutin, quercetin-β-D-glucoside, quercitrin, isorhamnetin-3-*O*-glucoside, diometin and chrysoeriol were confirmed by comparison with analytical standards. These findings are consistent with previous studies, which have reported a high diversity of glycosylated flavonoids in date fruit. Farag et al. (2014) identified 20 flavonoids in several Egyptian cultivars, mainly flavonols and flavones. While Nematallah et al. (2018) detected nineteen flavonoids in Ajwa dates at the Tamr stage. In Mature Deglet Noor dates, 13 glycosides of luteolin, quercetin, and apigenin have also been reported. Notably, some authors have reported the unusual occurrence of flavonoid sulfates in dates, a feature rarely described in foods (Hong et al., 2006).

The highest proportion of (poly)phenols was found in the insoluble-bound fraction, which accounted for approximately 87 % of the total (poly)phenol content, mainly due to the contribution of proanthocyanidins 4 and 5. Similar findings were reported by Hammouda et al. (2013), who observed that procyanidin polymers represented about 80 % of the total (poly)phenols in Deglet Nour dates at the Tamar stage. In the present work, the total soluble-free (poly)phenol content in Confitera date paste was near 8.5 mg/100 g fw, a value around seven times lower than reported by Khallouki et al. (2018) for fully ripe Medjool dates at the Tamar stage (61.3 mg/100 g). Other studies have reported values ranging from 10.9 to 42.3 mg/100 g dw in pulp (without peel) of 17 Moroccan cultivars at the Tamar stage (Alahyane et al., 2019), and from 25.1 to 33.9 mg/100g dw in whole Boufeggous and Mejhoul dates (Noutfia et al., 2025). These discrepancies can be largely attributed to differences in postharvest processing. Specifically, Confitera date analyzed in the present study was not subjected to sun-drying, a common preservation practice in date fruits (Jaouhari et al., 2024). Consequently, the paste retained a moisture content of approximately 48 %,

Table 1
Specification to (poly)phenol compounds detected in date palm fruit paste cv. Confitera.

No.	rt (min)	Fr.	λmax (nm)	Tentative identification	Standard use to quantified
1	7.9	B	236 280	Flavan-3-ol	Catechin
2	11.2	F	242 294sh 318	Hydroxybenzoic derivative 1	Vanillin
3	11.8	F	244 292sh 314	Caffeic acid glycoside	Caffeic acid
4	12.1	F	242 290sh 320	Caffeoylshikimic acid glycoside	Chl
5	12.7	F	242 292 318	Hydroxybenzoic derivative 2	Vanillin
6	12.7	B	236 274 406 474	Anthocyanin derivative 1	Pel-3-glu
7	12.9	F	242 290sh 322	Caffeoylshikimic acid 1	Chl
8	13.7	B	236 280 310	Vanillin glycoside 1	Vanillin
9	14.7	F	254 348	Quercetin triglycoside	Rutin
10	14.8	B	236 320 458	Unknow	–
11	14.9	F	246 292sh 326	Chlorogenic acid*	Chl
12	15.2	B	236 280 310	Vanillin glycoside 2	Vanillin
13	15.3	B	236 280	Catechin *	Catechin
14	15.6	B	236 280	Proantochyanidin 1	PAC B2
15	17.3	F	266 338	Apigenin glycoside	Api-7-glu
16	17.3	F	244 284sh 324	Caffeoylshikimic acid 2	Chl
17	17.6	F	242 290sh 324	Caffeoylshikimic acid 3	Chl
18	17.8	B	236 280	Proanthocyanindin 2	PAC B2
19	17.9	B	236 280	Epicatechin*	Epicatechin
20	18.1	F	242 300sh 326	Caffeic acid*	Caffeic acid
21	18.2	B	236 274 414 474	Anthocyanin derivative 2	Pel-3-glu
22	18.4	B	236 280	Proanthocyanidin 3	PAC B2
23	18.9	F	244 sh286 322	Caffeoylshikimic acid 4	Chl
24	19.4	F	244 sh286 324	Caffeoylshikimic acid 5	Chl
25	19.6	B	240 280	Proantochyanidin 4	PAC B2
26	20.0	F	254 358	Quercetin diglycoside 1	Rutin
27	20.7	F	258 360	Quercetin diglycoside 2	Rutin
28	21.0	B	238 272 416 472	Anthocyanidin derivative	Pel-3-glu
29	21.1	B	236 278	Proantochyanidin 5	PAC B2
30	22.1	F	256 358	Quercetin diglycosilate 3	Rutin
31	22.3	F	256 358	Quercetin-3-rutinoside (Rutin)*	Rutin
32	22.5	B	236 292sh 330	Flavanone hexoside	Naringin
33	23.7	F	256 264sh 362	Quercetin-3-β-D-glucoside*	Que-3-glu
34	24.1	B	238 270 332 482	Unknow	–
35	24.1	B	240 278	Catechin-3-gallate*	Cat-3-gal
36	24.6	F/B	246 286sh 324	Ferulic acid*	Ferulic acid
37	24.9	F	256 358	Quercetin glycosilate 1	Que-3-glu
38	25.4	F	256 358	Quercetin glycosilate 2	Que-3-glu
39	25.9	F	264 354	Isorhamnetin-3-*O*-glucoside*	Iso-3-glu
40	26.0	F	254 266 350	Diosmetin-7-*O*-rutinoside (Diosmin)*	Diosmin
41	26.3	F	254 356	Quercetin-3-rhamnoside (Quercitrin)*	Quercitrin
42	26.9	B	236 276 452	Anthocyanin derivative 4	Pel-3-glu
43	28.1	F	254 362	Isorhamnetin glycoside	Iso-3-glu
44	28.7	F	252 268 348	Chrysoeriol glycoside 1	Diosmin
45	29.4	F	252 268 346	Chrysoeriol glycoside 2	Diosmin
46	32.9	B	242 300sh 326	Caffeic acid derivative	Caffeic acid
47	33.8	F/B	256 370	Quercetin*	Quercetin[a]
48	38.0	F	252 268 348	Chrysoeriol*	Chrysoeriol
49	38.5	F	253 266 348	Luteolin mutilated	Diosmin
50	38.7	B	266 366	Kaempferol*	Kaempferol

rt: retention time; Fr.: Fraction; B: Insoluble-bound; F: Soluble-free.
Chl: Chlorogenic acid; Proantochyanidin B2: PAC B2; Apigenin-7-glucoside: Api-7-glu; Isorhamnetin-3-*O*-glucoside: Iso-3-glu; Quercetin-3-β-D-glucoside: Que-3-glu; Pelargonidin 3-*O*-β-glucopyranoside: Pel-3-glu; Cat-3-gal: Catechin-3-gallate.
[a] Compound confirmed by standard.

compared with the ~17 % reported for sun-dried Medjool dates (Eid et al., 2013).

3.2. (Poly)phenol profile on cheese goat

As expected, no (poly)phenols were detected in the control goat cheese (prepared without date paste). In contrast, a total of 22 compounds were quantified in the DPC-4 and DPC-8 formulations, including seventeen in the soluble-free fraction forms and five in the insoluble-bound fractions. (Table 3). Consistent with the profile of the date paste, chrysoeriol glycoside 1, caffeic acid, and caffeoylshikimic acid 1 were the predominant soluble (poly)phenols in both date-paste-enriched fresh goat cheeses (Table 3).

The recovery of total soluble-free and insoluble-bound (poly)phenols in both DPC-4 and DPC-8 was approximately 59–60 % and 11–19 %, respectively (Table 3). Flavonols were retained in the cheese matrix to a greater extent than flavones and hydroxycinnamic acids, which showed retention around 50 %. As a result, the enriched cheeses exhibited a higher relative proportion of flavonols than the original date paste, where soluble hydroxycinnamic acids were the predominant group. Mangiapelo et al. (2025) similarly reported high stability of glycosylated quercetin but a reduction of chlorogenic acid, attributed to enzymatic hydrolysis by cinnamoyl esterase converting it to caffeic acid. Additionally, chlorogenic acid has been shown to form non-covalent interactions with β-lactoglobulin, a major whey protein (Ren et al., 2023), which may further influence its distribution between curd and whey. Other studies reported reductions in hydroxycinnamic acids in dairy milk, such as Helal and Tagliazucchi (2018), who observed decreased hydroxycinnamic acid levels in coffee-fortified yogurt during storage. These differences may be attributed to variations in solubility and the affinity of individual (poly)phenols to interact with milk proteins. Furthermore, the addition of date palm to goat cheese fresh decreased the cheese yield in a concentration dependent manner (Muñoz-Bas et al., 2024b). Indicating that date palm affects curd formation and part of date palm added was lost on drained curd.

Table 2
Individual (poly)phenol content and total amount of compounds (μg/g f.w) from undigested and digested date palm paste variety Confitera at the Tamar stage.

Subfamily	Compounds		Undigested	Digested
Hydroxybenzoic acid derivatives	Hydroxybenzoic derivative 1	F	0.9 ± 0.1[a]	0.8 ± 0.0[a]
	Hydroxybenzoic derivative 2	F	2.5 ± 0.2[a]	2.7 ± 0.1[a]
	Vanillin glycoside 1	B	0.5 ± 0.1[b]	3.9 ± 0.4[a]
	Vanillin glycoside 2	B	3.1 ± 0.4[b]	22.7 ± 2.8[a]
Hydroxycinnamic acids and derivatives	Caffeic acid glycoside	F	1.2 ± 0.1[a]	0.7 ± 0.1[b]
	Caffeoylshikimic acid glycoside	F	7.2 ± 0.8[a]	7.3 ± 0.9[a]
	Caffeoylshikimic acid 1	F	8.5 ± 0.6[a]	5.0 ± 0.2[b]
	Chlorogenic acid	F	3.2 ± 0.3[a]	2.7 ± 0.2[a]
	Caffeoylshikimic acid 2	F	1.1 ± 0.1	nd
	Caffeoylshikimic acid 3	F	3.0 ± 0.3	nd
	Caffeic acid	F	8.8 ± 0.9[b]	11.7 ± 0.5[a]
	Caffeoylshikimic acid 4	F	3.5 ± 0.2[b]	4.3 ± 0.3[a]
	Caffeoylshikimic acid 5	F	3.6 ± 0.3[b]	9.8 ± 1.1[a]
	Ferulic acid	F	0.4 ± 0.0[b]	0.6 ± 0.1[a]
		B	5.9 ± 0.5[b]	15.4 ± 0.4[a]
	Caffeic acid derivative	B	nd	1.2 ± 0.1
Flavonols	Quercetin triglycoside	F	nd	0.6 ± 0.1
	Quercetin diglycoside 1	F	nd	0.6 ± 0.0
	Quercetin diglycoside 2	F	nd	0.4 ± 0.0
	Quercetin diglycoside 3	F	1.4 ± 0.2[b]	1.6 ± 0.1[a]
	Quercetin-3-rutinoside	F	1.7 ± 0.1[a]	1.5 ± 0.0[a]
	Quercetin-3-β-D-glucoside	F	1.9 ± 0.2[a]	1.2 ± 0.2[b]
	Quercetin glycoside 1	F	2.1 ± 0.0[a]	2.3 ± 0.3[a]
	Quercetin glycoside 2	F	3.6 ± 0.3[a]	2.8 ± 0.3[a]
	Isorhamnetin-3-*O*-glucoside	F	0.6 ± 0.1[a]	0.3 ± 0.1[b]
	Quercetin-3-rhamnoside	F	1.3 ± 0.1[a]	0.9 ± 0.3[a]
	Isorhamnetin glycoside	F	1.6 ± 0.2[a]	0.3 ± 0.1[b]
	Quercetin	F	0.6 ± 0.1	nd
		B	1.2 ± 0.3[a]	1.5 ± 0.3[a]
	Kaempferol	B	nd	0.2 ± 0.0
Flavones	Apigenin glycoside	F	1.1 ± 0.1[a]	1.0 ± 0.1[a]
	Diosmetin 7-*O*-rutinoside	F	4.4 ± 0.3[a]	2.3 ± 0.2[b]
	Chrysoeriol glycoside 1	F	16.6 ± 1.2[a]	4.6 ± 0.7[b]
	Chrysoeriol glycoside 2	F	1.4 ± 0.0[b]	2.3 ± 0.5[a]
	Luteolin metilated	F	1.6 ± 0.1	nd
	Chrysoeriol	F	2.8 ± 0.3	nd
Flavan-3-ols	Flavan-3-ol derivative	B	11.4 ± 3.1[b]	40.7 ± 5.4[a]
	Catechin	B	35.3 ± 2.3[a]	21.8 ± 5.7[b]
	Proantocyanidin 1	B	14.9 ± 2.5[a]	16.6 ± 0.6[a]
	Proanthocyanidin 2	B	19.0 ± 1.9[a]	12.4 ± 0.3[b]
	Proanthocyanidin 3	B	19.6 ± 3.5	nd
	Epicatechin	B	10.8 ± 0.7[b]	34.9 ± 2.0[a]
	Proantochyanidin 4	B	61.8 ± 5.2[a]	31.5 ± 3.0[b]
	Proantochyanidin 5	B	252 ± 79.2	nd
	Catechin-3-gallate	B	17.4 ± 2.1	nd
Other flavonoids	Anthocyanin derivative 1	B	nd	11.0 ± 0.5
	Anthocyanin derivative 2	B	0.3 ± 0.0[b]	2.1 ± 0.1[a]
	Anthocyanin derivative 3	B	1.4 ± 0.3[b]	38.1 ± 0.3[a]
	Anthocyanin derivative 4	B	nd	1.9 ± 0.4
	Flavanone	B	13.9 ± 1.2[b]	112 ± 8.1[a]
	Total, (poly)phenols	F	86.9 ± 2.1[a]	68.8 ± 1.6[b]
		B	468 ± 85.1[a]	367.4 ± 18.2[a]
	Total, Hydroxycinnamic acids	F	40.9 ± 2.5[a]	42.8 ± 1.5[a]
		B	5.9 ± 0.5[b]	16.6 ± 0.4[a]
	Total, Hydroxybenzoic acids	F	3.5 ± 0.3[a]	3.5 ± 0.1[a]
		B	3.4 ± 0.5[b]	26.6 ± 2.9[a]
	Total, Flavonols	F	14.7 ± 1.2[a]	12.4 ± 1.4[a]
		B	1.2 ± 0.3[a]	1.6 ± 0.2[a]
	Total, Flavones	F	27.9 ± 1.4[a]	10.1 ± 1.1[b]
	Total, Flavan-3-ols	B	442 ± 83.3[a]	158 ± 11.9[b]

nd: Not detected. Different letters in the same row indicated significant differences according to Tuckey's post hoc test ($p < 0.05$).

Regarding the recovery index of individual (poly)phenols, the most remarkable value was observed for ferulic acid, with a recovery index exceeding 800 % in both enriched cheeses (Table 2). This phenomenon could be attributed to the release of conjugated ferulic acid during cheese manufacture, likely resulting from its association with other macronutrients, such as proteins. Indeed, in date seeds, caffeic acid, ferulic acid, p-coumaric acid, and quercetin have been reported at significantly higher concentrations in soluble-conjugated fractions than in their soluble-free forms (Lucas-González et al., 2023). Furthermore, the mediated enzymatic hydrolysis can also release ferulic acid that found conjugated with caffeic acid or other quinic acid derivatives (Mangiapelo et al., 2025).

Many authors who enriched dairy products with plant extracts have pointed out that differences in recovery are related to the varying binding affinities of individual polyphenols to milk proteins. For example, on cinnamon extract-fortified yogurt the recovery of kaempferol was 21 % while the recovery of quercetin was 55 % (Helal & Tagliazucchi, 2018). Trigueros et al. (2014) reported that, among phenolic compounds from pomegranate juice present on yogurt, ellagic acid and delphinidin-3,5-*O*-diglucoside exhibited the lowest affinity for binding to milk proteins. Furthermore, they highlighted that, in general, monoglucosides of flavonoids show stronger binding affinities to milk proteins than their polyglucoside forms. This phenomenon could explain the differences in recovery index observed in the present study among flavanols, such as quercetin-3-β-D-glucoside, isorhamnetin glycoside,

Table 3
(Poly)phenol profile (μg/g f.w) of undigested and digested enriched date palm-goat fresh cheeses and the theorical retention index after manufacturing process.

		Undigested DPC-4	Digested DPC-4	Undigested DPC-8	Digested DPC-8	RI (%) DPC-4	RI (%) DPC-8
Hydroxybenzoic derivative 1	F	2.2 ± 0.2^{b}	nd	5.9 ± 0.4^{a}	nd	59.8 ± 5.9^{a}	81.6 ± 6.0 ^{b}
Hydroxybenzoic derivative 2	F	4.2 ± 0.5^{b}	nd	8.5 ± 0.4^{a}	nd	42.5 ± 4.9^{a}	43.0 ± 2.1^{a}
Vanillin glycoside 1	B	4.5 ± 0.3^{c}	18.9 ± 2.7^{b}	4.9 ± 0.9^{c}	30.3 ± 2.9^{a}	219 ± 16.0^{a}	120.2 ± 22.7^{b}
Vanillin glycoside 2	B	33.6 ± 10.4^{c}	103 ± 3.6^{b}	32.4 ± 14.9^{c}	241.3 ± 3.5^{a}	274 ± 84.5^{a}	132 ± 60.6^{a}
Caffeoylshikimic acid glycoside	F	17.4 ± 2.1^{b}	3.5 ± 0.1^{c}	36.0 ± 3.2^{a}	4.0 ± 0.1^{c}	60.6 ± 7.3^{a}	62.8 ± 5.6^{a}
Chlorogenic acid	F	2.9 ± 0.7^{b}	nd	10.3 ± 0.0^{a}	nd	22.9 ± 5.4^{b}	40.1 ± 0.1^{a}
Caffeic acid	F	32.9 ± 0.4^{b}	16.0 ± 0.2^{c}	54.5 ± 2.8^{a}	17.5 ± 0.2^{c}	93.7 ± 1.1^{a}	77.6 ± 4.0^{b}
Caffeoylshikimic acid 5	F	19.5 ± 4.0^{b}	4.7 ± 0.5^{c}	49.3 ± 6.6^{a}	10.5 ± 0.7^{c}	134 ± 27.6^{a}	170 ± 22.8^{a}
Ferulic acid	F	16.3 ± 2.9^{b}	nd	27.2 ± 0.1^{a}	nd	1064 ± 192.8^{a}	892 ± 2.4^{a}
	B	5.4 ± 0.4^{c}	55.8 ± 3.3^{b}	10.5 ± 0.1^{c}	119 ± 3.6^{a}	22.9 ± 1.6^{a}	22.5 ± 0.2^{a}
Quercetin diglycoside 3	F	8.1 ± 0.9^{b}	nd	15.7 ± 1.7^{a}	nd	141.7 ± 15.7^{a}	137 ± 15.1^{a}
Quercetin-3-rutinoside (Rutin)	F	9.2 ± 1.8^{b}	nd	15.4 ± 1.5^{a}	nd	140 ± 27.9^{a}	116.4 ± 11.4^{a}
Quercetin-3-β-D-glucoside	F	6.3 ± 0.2^{a}	nd	9.6 ± 2.3^{a}	nd	82.7 ± 3.0^{a}	63.7 ± 15.0^{a}
Quercetin glycoside 1	F	12.9 ± 1.9^{b}	3.5 ± 0.1^{c}	24.3 ± 1.5^{a}	4.9 ± 0.2^{c}	156 ± 23.2^{a}	147 ± 9.2^{a}
Quercetin glycoside 2	F	16.7 ± 3.6^{b}	nd	29.9 ± 0.9^{a}	nd	116 ± 24.7^{a}	104 ± 3.3^{a}
Quercetin-3-rhamnoside (Quercitrin)	F	4.9 ± 0.4^{b}	nd	8.5 ± 0.3^{a}	nd	97.5 ± 7.3^{a}	84.6 ± 3.4^{a}
Isorhamnetin glycoside	F	2.8 ± 0.2^{b}	nd	5.7 ± 1.1^{a}	nd	43.4 ± 3.1^{a}	45.1 ± 8.4^{a}
Apigenin glycoside	F	4.0 ± 0.3^{b}	nd	7.9 ± 0.5^{a}	nd	88.3 ± 7.5^{a}	87.6 ± 5.7^{a}
Diosmetin 7-*O*-rutinoside (Diosmin)	F	10.7 ± 0.8^{b}	nd	25.7 ± 0.5^{a}	nd	61.0 ± 4.5^{b}	73.3 ± 1.5^{a}
Chrysoeriol glycoside 1	F	34.8 ± 5.3^{b}	nd	70.2 ± 8.7^{a}	nd	52.3 ± 7.9^{a}	52.8 ± 6.1^{a}
Flavanone	B	nd	870 ± 8.9^{b}	nd	1151 ± 70.5^{a}	–	–
Proantochyanidin 4	B	252 ± 15.9^{a}	nd	285 ± 9.6^{a}	nd	102 ± 6.4^{a}	57.5 ± 1.9^{b}
Anthocyanin derivative 1	B	nd	tr	nd	tr	–	–
Anthocyanin derivative 3	B	21.3 ± 0.1^{a}	65.6 ± 14.8^{a}	30.2 ± 6.4^{a}	98.2 ± 50.7^{a}	374 ± 2.2^{a}	264 ± 55.9^{a}
Anthocyanin derivative 4	B	nd	38.3 ± 27.1	nd	40.1 ± 0.7	–	–
Total (poly)phenols	F	206 ± 24.6^{b}	27.7 ± 0.6^{c}	405 ± 23^{a}	36.9 ± 1.0^{c}	59.2 ± 7.1^{a}	58.2 ± 3.4^{a}
	B	316 ± 10.7^{c}	1220 ± 64.1^{b}	363 ± 12.3^{c}	1700 ± 32.7^{a}	16.9 ± 0.6^{a}	9.7 ± 0.3^{b}
Total Hydroxycinnamic acids	F	88.9 ± 9.5^{b}	24.2 ± 0.6^{c}	177 ± 10.7^{a}	32. ± 0.8^{c}	54.4 ± 5.8^{a}	54.2 ± 3.3^{a}
	B	5.4 ± 0.4^{c}	55.8 ± 3.3^{b}	10.5 ± 0.1^{c}	119 ± 3.6^{a}	22.9 ± 1.6^{a}	22.5 ± 0.2^{a}
Total Hydroxybenzoic acids	F	6.4 ± 0.6^{b}	nd	14.4 ± 0.7^{a}	nd	47.1 ± 4.7^{a}	53.3 ± 2.6^{a}
	B	38.0 ± 10.0^{c}	121.7 ± 5.9^{b}	37.3 ± 15.6^{c}	272 ± 6.0^{a}	266 ± 70.2^{a}	131 ± 54.7^{a}
Total Flavonols	F	60.9 ± 8.4^{b}	3.5 ± 0.1^{c}	109 ± 7.7^{a}	4.9 ± 0.2^{c}	103.4 ± 14.2^{a}	92.7 ± 6.5^{a}
Total Flavones	F	49.5 ± 6.4^{b}	nd	104 ± 8.3^{a}	nd	44.3 ± 5.7^{a}	46.5 ± 3.7^{a}
Total Flavan-3-ols	B	252 ± 15.9^{a}	nd	285 ± 9.6^{a}	nd	14.2 ± 0.9^{a}	8.1 ± 0.3^{b}

Nd: not detected; tr: traces; DPC-4: fresh goat cheese with 4 % addition of date palm paste; DPC-8: fresh goat cheese with we8% addition of date palm paste; RI: Retention index; F: Soluble-free fraction: B: Insoluble-bound fraction.
Regarding undigested and digested samples or recovery index values different letters in the same row indicated significant differences according to Tuckey's post hoc test ($p < 0.05$).

and quercetin-3-rutinoside (Table 2).

In the insoluble-bound fraction, only proanthocyanidin 4 was detected among the nine flavan-3-ols in date paste. In contrast, two vanillin derivatives—originally present in smaller amounts than catechin and other flavan-3-ols—were found in the enriched cheese, along with ferulic acid and anthocyanin derivatives. The strong binding affinity of catechin and epicatechin with milk protein have been previously reported on mixture to milk and chocolate and tea and milk (Oliveira et al., 2015). Similar losses of insoluble-bound flavan-3-ols have been previously reported in other food matrices; for example, in pork liver pâté enriched with persimmon flour, only one of ten flavan-3-ols from the flour was detected in the enriched product (Lucas-González et al., 2021).

3.3. Stability of (poly)phenols from date palm paste after in vitro gastrointestinal digestion

The (poly)phenol profile of date palm underwent noticeable modifications following the gastrointestinal digestion process. Of the 27 soluble (poly)phenols detected in undigested date palm, five—caffeoylshikimic acids 2 and 3, quercetin, methylated luteolin, and chrysoeriol—, were no longer detected, likely due to their initial low concentration. Conversely, certain flavonols, including quercetin triglycoside and quercetin diglycosides 1 and 3, were only detected after *in vitro* digestion, indicating that the digestive process facilitated their release.

A similar trend was observed in the insoluble-bound fraction. Proanthocyanidins 3 and 5, along with catechin-3-gallate, were no longer detected after digestion. Instead, new compounds emerged during the *in vitro* digestion process, including a hydroxycinnamic acid derivative, kaempferol, and two anthocyanin derivatives.

Nevertheless, aside from the compounds that disappeared after digestion, (poly)phenols in date palm paste, generally exhibited high stability throughout the *in vitro* digestion process. Most compounds, such as caffeoylshikimic acid glycoside, chlorogenic acid, quercetin-3-rutinoside, quercetin-3-rhamnoside, and apigenin glycoside, showed no significant changes in concentration before and after digestion ($p > 0.05$). In contrast, some soluble compounds, including caffeic acid, quercetin diglycoside 3, caffeoylshikimic acids 4 and 5, and chrysoeriol glycoside 2, were detected at higher concentrations after digestion ($p < 0.05$). Certain insoluble-bound compounds, such as ferulic acid, epicatechin, flavanone and anthocyanin 1 and 4, were also detected at significantly higher levels than in undigested samples, ($p < 0.05$), suggesting enhanced release during digestion. Conversely, some soluble-free compounds decreased after digestion, as expected. Notably, chrysoeriol glycoside 1 and caffeoylshikimic acid 1, which were predominant in undigested samples, showed a marked and significant decrease after *in vitro* digestion ($p < 0.05$).

These findings are consistent with earlier studies on date fruit and seeds. Djaoudene et al. (2021) evaluated freeze-dried extracts of fruit pulp and seeds from eight Algerian date cultivars and found that *in vitro* digestion reduced pulp flavonols but increased caffeic acid, while seed extracts showed marked increases in phenolic acids and flavonoids across all cultivars. Likewise, Kamal et al. (2023) analyzed digested samples from four date varieties (Safawi, Khalas, Khudri, and Booman), and detected rutin, caffeic acid, and 4-hydroxybenzoic only after digestion. They also reported substantial increases in 1,2-dihydroxybenzoic and reductions in catechin and *p*-coumaric acid during the intestinal

phase in specific varieties. Overall, these studies show that the stability of polyphenols in date pulp and seed extracts is strongly affected by digestion, with outcomes depending on compound type and cultivar.

The different patterns in (poly)phenol stability can be explained by several interconnected factors. First, the increase in soluble-free (poly) phenols may result from the presence of soluble-conjugate forms (esterified, etherified or glycosylated) that were not extracted initially methodology but became released into the digestive medium through enzymatic hydrolysis. Indeed, in date seed, caffeic acid, ferulic acid, *p*-coumaric acid, and quercetin have been reported at significantly higher concentrations in soluble-conjugated fractions than in their soluble-free forms ($p < 0.05$) (Lucas-González et al., 2023). Similar trends have been observed for caffeic acid and chlorogenic acid in calafate (*Berberis microphylla*) byproducts, and for quercetin and rutin in araticum (*Annona crassiflora* Mart.), where esterified forms predominate (Arruda et al., 2018; Concepción-Alvarez et al., 2025). Second, the protective effect of the food matrix must be taken into account. Date palm fruit paste is a minimally processed product rich in dietary fiber and sugars, which may help safeguard (poly)phenols during gastrointestinal transit. Peters et al. (2010) used both *in vitro* and *in vivo* approaches, to show that sucrose can interfere with the binding of flavan-3-ols to other green tea components, such as caffeine, thereby enhancing catechin solubility and improving their bioaccessibility. Moreover, dietary fiber may physically entrap soluble (poly)phenols, which helps to stabilize (poly) phenols throughout digestion (Viuda-Martos et al., 2018). Finally, differences in (poly)phenol stability under alkaline intestinal conditions, along with potential interactions with bile salts, may further explain the compound-specific variability observed in this study (Lucas-González et al., 2018).

3.4. (Poly)phenols stability after in vitro gastrointestinal digestion of date palm paste–enriched fresh goat cheese

As observed for date palm paste alone, the (poly)phenolic profile of the enriched date palm paste–fresh goat cheese changed markedly after *in vitro* gastrointestinal digestion (Table 3). Of the seventeen soluble (poly)phenols present in the undigested P4 and P8 samples, only four were detected post-digestion: caffeic acid, caffeoylshikimic acid glycoside, caffeoylshikimic acid 5, and quercetin glycoside 1. All of these showed a significant decrease in their concentration after digestion compared with undigested sample ($p < 0.05$). Notably, chrysoeriol glycoside 1, the predominant soluble-free flavonoid in the undigested enriched date palm paste–fresh goat cheeses, was no longer detectable after digestion. This trend mirrors that observed in date paste, where chrysoeriol 1 dramatically decreased after digestion, and underscores the marked instability of this compound during both cheese manufacturing and *in vitro* digestion.

In the insoluble-bound fractions of the enriched date palm paste–-fresh goat cheeses, most (poly)phenols were present at significantly higher concentrations after digestion than in the undigested samples ($p < 0.05$), except for proanthocyanidin 4, which disappeared during the intestinal phase. Additionally, three new compounds, a flavone, and anthocyanin derivatives 1 and 4—were detected in both digested cheeses but were absent in the undigested samples (Table 3).

After gastrointestinal digestion, the only notable differences in (poly) phenol concentrations between the two enriched date palm paste–fresh goat cheeses (DPC-4 and DPC-8) were found in the insoluble-bound phenolic acids. DPC-8 showed significantly higher levels of these compounds. All other soluble and insoluble-bound phenolics were present at comparable concentrations in both cheeses without statistically significant difference ($p > 0.05$).

Comparable studies have examined enriched date seeds extract yogurt and seed extract-enriched bread subjected to *in vitro* gastrointestinal digestion. For the enriched bread, the findings aligned with those of the present study: (poly)phenol levels declined after digestion, and neither flavonols nor flavones were detected. In contrast, enriched yogurt demonstrated high (poly)phenols stability after the intestinal phase (Hilary et al., 2020). This discrepancy may be related to the fact that flavan-3-ols were the only (poly)phenolic compounds detected in yogurt, and these may exhibit different stability profiles compared with flavones and flavonols, which were the only flavonoids detected in the soluble-free fraction of the enriched date paste cheese. López-Astorga et al. (2025) added microencapsulated grape pomace extracts to Greek-style yogurt and observed less stability of phenolic acids than flavonoids. In line with the present study, López-Astorga et al. (2025) also highlighted that the nature of the (poly)phenols strongly influences their release from the food matrix and their stability during digestion.

3.5. Bioaccessibility and colon available index of date palm paste and enriched-goat fresh cheese

The bioaccessibility of (poly)phenols refers to the proportion of compounds that are released from the food matrix during digestion and become for transformation and absorption into the bloodstream. In contrast, the colon available index represents the fraction of (poly) phenols that remain bound to the food matrix and reach the colon, where they can be metabolized by the gut microbiota (Lucas-González et al., 2021).

Fig. 1 presents the bioaccessibility and colon-available index of the total (poly)phenol content in date palm paste, DPC-4, and DPC-8. Date palm paste exhibited the highest (poly)phenol bioaccessibility index ($p < 0.05$), whereas DPC-8 showed the highest colon-available index ($p < 0.05$). The low total (poly)phenol bioaccessibility of date palm paste contrasts with the high bioaccessibility values observed for several soluble-free individual (poly)phenols, which ranged from approximately 50–260 % (Fig. 2). This discrepancy arises because insoluble bound (poly)phenols represented the largest fraction in the date palm paste and were not released into the chyme solution during *in vitro* gastrointestinal digestion.

These results highlight the strong influence of the food matrix on the bioaccessibility and colon available index of (poly)phenols. The complex cheese matrix drastically reduced the release or stability of (poly)phenols during gastrointestinal digestion. In contrast, when date paste was digested alone, its main (poly)phenols showed high stability and bioaccessibility (Fig. 2). Similar findings have been previously reported, where the bioaccessibility of (poly)phenols from tomatoes and peppers was higher than that of processed foods prepared with them as main ingredients (Lucas-González et al., 2023).

Regarding the four bioaccessible compounds detected in goat cheese enriched with date palm paste, caffeoylshikimic acid glycoside, caffeoylshikimic acid, caffeic acid, and quercetin glycoside 1 (Fig. 3), the differences compared with date paste were pronounced. In the date paste, those compounds showed bioaccessibility index values equal to or exceeding 100 %. In contrast, in both enriched cheese samples (DPC-4 and DPC-8), the bioaccessibility values ranged from 11.10 % to 48.78 %. Among them, caffeic acid was reported the highest bioaccessibility index. Furthermore, no significant differences were found between the two enriched cheese samples ($p > 0.05$), indicating that the low bioaccessibility of date (poly)phenols within the cheese matrix was independent of their initial concentration. López-Astorga et al. (2025) reported higher percentages of compound release in yogurt fortified with 6 % microencapsulated grape pomace extract than those observed in the present study, with values ranging from 11.3 % to 10 277 %. In their case, the degradation of anthocyanins was responsible for the marked increase in syringic acid (10 277 %). As observed in the present work, they also reported variable release of quercetin derivatives: some were no longer detected after digestion, whereas others—such as quercetin dihydrate and isorhamnetin 3-*O*-glucoside—showed release indices of 76.2 % and 80.4 %, respectively. Other authors have subjected dairy products enriched with (poly)phenol -rich extracts to *vitro* digestion; however, phenolic stability in those studies was assessed using colorimetric methods, which offer less accurate results (Coelho et al.,

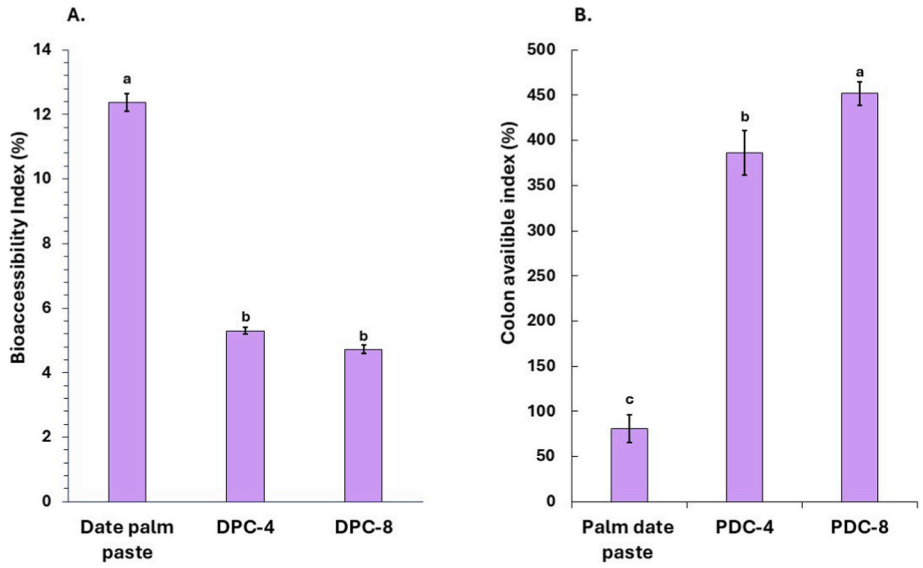


Fig. 1. A) Total bioaccessibility index and B) Colon available index of total (poly)phenols of date palm paste, fresh goat cheese enriched with 4 % of date palm paste (DPC-4) and fresh goat cheese enriched with 8 % of date palm paste (DPC-8).

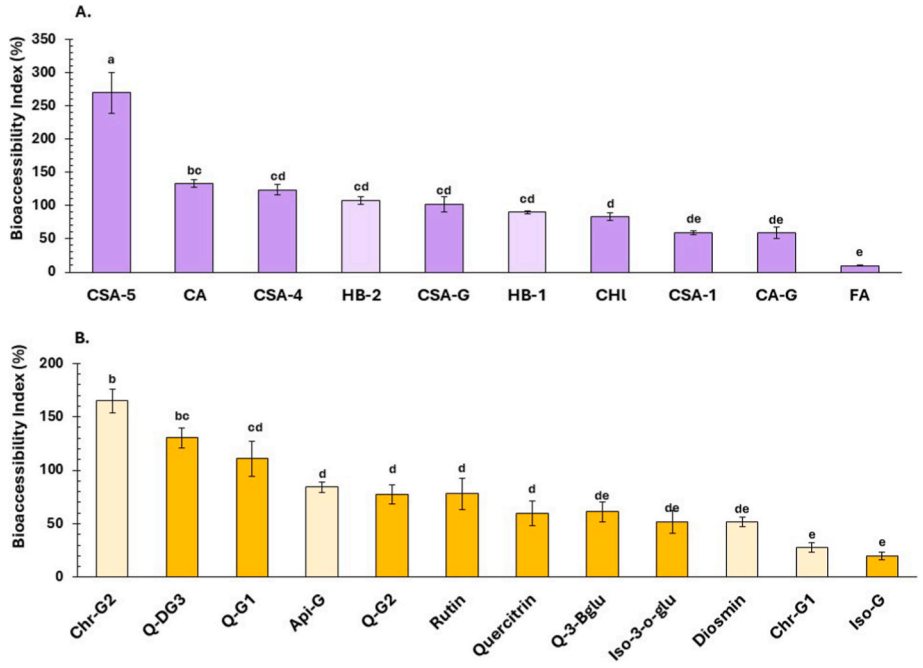


Fig. 2. A) Bioaccessibility index of individual hydroxycinnamic and hydroxybenzoic acids and derivatives of date palm paste. B) Bioaccessibility index of individual Flavonols and flavones from date palm paste.
CSA-5: Caffeoylshikimic acid 5; CA: Caffeic acid; CSA-4: Caffeoylshikimic acid 4; HB-2: Hydroxybenzoic acid derivative 2; CSA-G: Caffeoylshikimic acid glycoside; HB-1: Hydroxybenzoic acid derivative 1; CHl: Chlorogenic acid; CSA-1: Caffeoylshikimic acid 1; CA-G: Caffeic acid glycoside; Fa: Ferulic acid. Chr-G2: Chrysoeriol glycoside 2; Q-DG3: Quercetin diglycoside 3; Q-G1: Quercetin glycoside 1; Api-G: Apigenin glycoside; Q-G2: Quercetin glycoside 2; Q-3-Bglu: Quercetin-3-β-D-glucoside; Iso-3-*O*-glu: Isorhamnetin-3-*O*-glucoside; Chr-G1: Chrysoeriol glycoside 1; Iso-G: Isorhamnetin glycoside.

2024; Zheng et al., 2024).

Concerning the colon available index of the individual insoluble-bound (poly)phenols (Fig. 4), different trends were observed among enriched goat fresh cheese compared with date paste. For vanillin glycosides 1 and 2, similar values were reported among all three samples ($p > 0.05$). In contrast, the colon available index of ferulic acid was lower in date paste than in both enriched goat cheese samples ($p < 0.05$). Conversely, in the case of anthocyanin derivative 3, the highest values were reported for date paste ($p < 0.05$). Therefore, although *in vitro* digestion process favored the increase of these compounds, the results revealed an influence of both the compound structure and the food matrix.

4. Limitations and future recommendations

Studies examining the effect of the food matrix on nutrient digestibility and the bioaccessibility of micronutrients and phytochemicals are still limited. The number of available publications remains low. Moreover, considering the current trend toward developing hybrid foods that combine animal-derived ingredients with plant-based

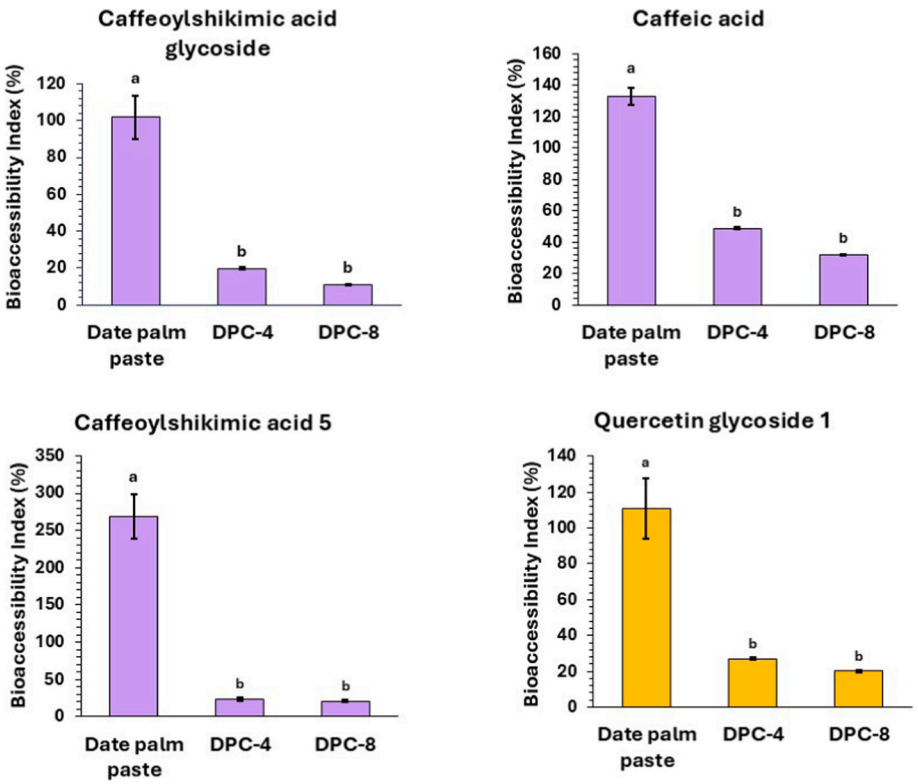


Fig. 3. Bioaccessibility of individual polyphenols of date palm paste, fresh goat cheese enriched with 4 % of date palm paste (DPC-4) and fresh goat cheese enriched with 8 % of date palm paste (DPC-8).

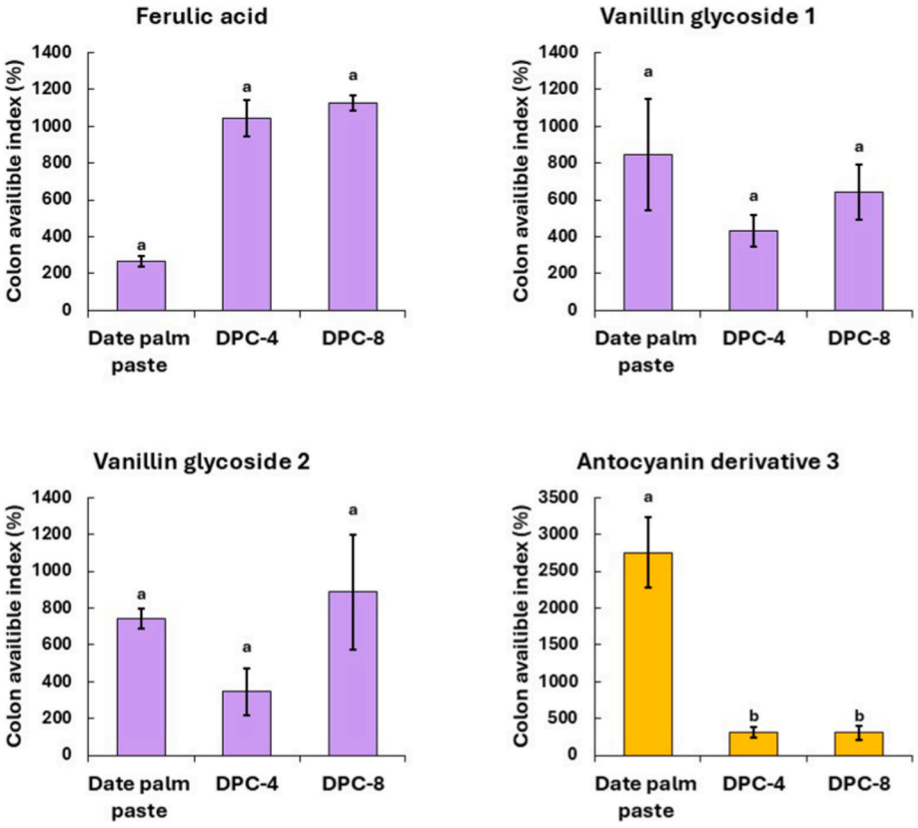


Fig. 4. Individual colon available index of date palm paste, fresh goat cheese enriched with 4 % of date palm paste (DPC-4) and fresh goat cheese enriched with 8 % of date palm paste (DPC-8).

components, it is increasingly necessary to assess their stability during manufactured and behavior during gastrointestinal digestion. The results on the present study indicate that on fresh cheese the study of (poly)phenols concentration on whey is necessary to improve understand the retention behavior and affinity by (poly)phenols with whey milk proteins.

Moreover, although the inclusion of bound (poly)phenols in the bioaccessibility assessment represents a significant advancement, given that these compounds are often overlooked, future research should also address other relevant fractions, such as conjugated (poly)phenol s, as well as the interactions between (poly)phenol s and proteins. Such analyses are necessary to investigate in depth the interactions, stability, and behavior of (poly)phenol s within their intrinsic matrix and in other food matrices, including dairy products. Furthermore, while *in vitro* digestion models provide a valuable tool for preliminary investigation, complementary *in vivo* studies are required to fully elucidate the physiological implications of these interactions in the human body.

5. Conclusions

Date palm paste (*Phoenix dactylifera*, Confitera variety) is a valuable source of (poly)phenols, including a substantial bound-insoluble fraction that is often underestimated in date fruits. *In vitro* gastrointestinal digestion showed that the cheese matrix markedly reduced the stability and bioaccessibility of these compounds, with recoveries below 40 % regardless of enrichment level. The digestion process also introduced substantial changes to the (poly)phenol profile, demonstrating that the composition of the raw material alone does not reflect the compounds ultimately available for absorption or colonic metabolism.

These findings indicate that incorporating date palm paste into dairy products can enhance their content of colon-available (poly)phenols but also highlight the need to improve food formulation strategies to better preserve (poly)phenol stability and release during digestion. Optimizing factors such as processing conditions, microencapsulation, or matrix interactions may increase the effectiveness of (poly)phenol delivery in hybrid dairy–plant foods. Further research is required to clarify the interactions between these compounds and the colonic microbiota and to better understand their potential health implications. In conclusion, the study pointed out that the food matrix plays a decisive role in modulating the release and stability of (poly)phenols during digestion.

CRediT authorship contribution statement

Raquel Lucas-González: Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Clara Muñoz-Bas:** Writing – original draft, Investigation, Formal analysis. **Nuria Muñoz-Tebar:** Methodology, Formal analysis. **José Ángel Pérez- Álvarez:** Investigation, Funding acquisition. **Manuel Viuda-Martos:** Writing – review & editing, Methodology, Investigation. **Juana Fernández-López:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119019.

Data availability

Data will be made available on request.

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