ESTRATEGIAS DE DESARROLLO DE LECHES FERMENTADAS CON POTENCIAL ANTIOXIDANTE Y EFECTO ANTI-OBESIDAD

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Estrategias de desarrollo de leches fermentadas con potencial antioxidante y efecto anti-obesidad

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Estrategias de desarrollo de leches fermentadas con potencial antioxidante y efecto anti-obesidad

Tesis doctoral realizada por Lorena Trigueros Medina, Licenciada en Ciencia y Tecnología de Alimentos, en el Departamento de Tecnología Agroalimentaria de la Universidad Miguel Hernández de Elche, para la obtención del grado de Doctor.

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Que la Tesis Doctoral titulada '**ESTRATEGIAS DE DESARROLLO DE LECHES FERMENTADAS CON POTENCIAL ANTIOXIDANTE Y EFECTO ANTI-OBESIDAD'** de la que es autora la Ingeniero Técnico Agrícola, y Licenciada en Ciencia y Tecnología de Alimentos **Lorena Trigueros Medina** ha sido realizada bajo la dirección de la **Dra. Esther Sendra Nadal,** profesora Titular de Universidad, la cual considero conforme en cuanto a forma y contenido para que sea presentada para su correspondiente exposición pública.

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Dña. Esther Sendra Nadal, Dra. en Veterinaria y Profesora Titular de Universidad del Departamento de Tecnología Agroalimentaria de la Universidad Miguel Hernández,

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Fdo.: Dra. Esther Sendra Nadal

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ESTRUCTURA DE LA TESIS

La presente Tesis Doctoral está basada en artículos científicos, tanto de investigación como bibliográficos, publicados o bajo revisión. Su estructura, por tanto, se adapta a la normativa interna de la Universidad Miguel Hernández para la 'Presentación de Tesis Doctorales con un Conjunto de Publicaciones'. Los diferentes capítulos en los que se ha organizado son detallados a continuación:

- **Abstract:** incluye un resumen global de los objetivos de la Tesis y de los resultados obtenidos.
- **Introducción:** consta de una revisión bibliográfica en relación a la situación actual del mercado de las leches fermentadas, tendencias de consumo así como de sus efectos beneficiosos. También se incluye una breve revisión sobre el origen, composición y propiedades saludables de cada uno de los ingredientes utilizados para en el desarrollo de las distintas experiencias: dátil, membrillo, granada y ácido linoleico conjugado.
- **Objetivos:** se detalla la hipótesis de trabajo así como el principal objetivo de la Tesis, junto con los objetivos secundarios.
- **Materiales y Métodos:** se presenta un resumen de los materiales y métodos empleados en la elaboración de las distintas leches fermentadas así como en la caracterización de las materias primas y los análisis realizados a las leches fermentadas enriquecidas.
- **Discusión general:** en este capítulo se muestra un resumen global de los resultados más relevantes obtenidos en los diferentes trabajos realizados, así como una discusión de los aspectos de más interés.
- **Conclusiones:** este capítulo recoge las conclusiones de todos los trabajos realizados.
- **Bibliografía:** recopila toda la bibliografía consultada en la introducción, materiales y métodos y en la discusión general.
- **Publicaciones:** este capítulo consta de todos los trabajos publicados (6) o bajo revisión (2), en su idioma original. Las primeras cinco publicaciones se centran en la caracterización química y determinación de las propiedades antioxidantes de las diferentes materias primas seleccionadas con potencial antioxidante así

como de la caracterización de los yogures enriquecidos: la primera, en la revista *LWT - Food Science and Technology* donde se recoge la caracterización de pasta, agua de escaldado y yogur enriquecido en membrillo; las dos siguientes publicadas en las revistas *Food Science and Technology* y *Food and Bioproducts and Processing* donde se caracterizan las pastas y agua de escaldado de los dos cultivares de dátiles empleados (Medjoul y Confitera) así como los yogures enriquecidos; a continuación la cuarta, publicada en la revista *Milchwissenschaft* donde se caracteriza el zumo de granada y el yogur enriquecido; y por último la quinta, con la que se cierra el grupo de publicaciones referente al primer bloque de resultados, en la revista *Journal of Agricultural and Food Chemistry* en la que se estudia el efecto de la interacción proteína-polifenol y se determinan las propiedades antioxidantes del yogur enriquecido en granada. El segundo grupo de publicaciones, correspondiente al segundo bloque de resultados, centrado en el estudio del ácido linoleico conjugado por su posible efecto anti-obesidad, incluye 3 artículos: el primero de ellos donde se realiza una profunda revisión bibliográfica sobre ingredientes alimentarios con potencial anti-obesidad publicado en la revista *Critical Reviews in Food Science and Nutrition*; el segundo donde se recogen los resultados en relación al nivel de CLA que presentan leches fermentadas comerciales y se encuentra en proceso de revisión en la revista *LWT - Food Science and Technology* y por último, el trabajo realizado sobre el efecto del uso de precursores para aumentar el nivel de CLA en leche de cabra que se encuentra en proceso de revisión en la revista *International Journal of Dairy Technology*.

RESUMEN

El desarrollo de leches fermentadas con propiedades saludables requiere de fundamento científico y viabilidad tecnológica. El presente trabajo aborda estrategias de desarrollo de leches fermentadas con potencial antioxidante, por incorporación de extractos vegetales, y de leches fermentadas con potencial efecto anti-obesidad por la potenciación del contenido en ácido linoleico conjugado (CLA).

Para la obtención de extractos de frutas se ha optado por aguas de escaldado de la industrialización de la pasta del dátil y del membrillo, y por el zumo de granada, todos ellos productos locales o del entorno cercano. Los dátiles y membrillos utilizados no eran aptos para su consumo en fresco y de este modo permiten explorar su aprovechamiento industrial, se obtuvo de ellos agua de escaldado y pasta.

Las pastas y aguas de escaldado de las industrias del dátil y membrillo pueden usarse como ingredientes en la formulación de alimentos funcionales por su contenido en antioxidantes naturales. La adición de aguas de escaldado de dátil y membrillo en la elaboración de yogur para reconstituir la leche provoca modificaciones en los yogures: el agua de dátil Medjoul aportó los mejores resultados tecnológicos y sensoriales, en cambio el agua de dátil Confitera y de membrillo, de mayor potencial antioxidante redujeron las poblaciones microbianas y las valoraciones sensoriales de los yogures. Se recomienda el uso de agua de escaldado de Medjoul en la elaboración de yogur por su sabor y aporte de azúcar.

Por su parte, el zumo de granada puede utilizarse hasta en un 40% en la formulación de leches fermentadas para obtener yogures de elevada capacidad antioxidante. Leche y zumo deben pasteurizarse separadamente para evitar la desestabilización de las proteínas de la leche. Estos yogures desarrollan menor acidez y son positivamente calificados en aceptación sensorial.

Es un hecho conocido que los compuestos fenólicos tienen una elevada afinidad por las proteínas, esto nos ha llevado a investigar las interacciones proteínas lácteas-polifenoles en los yogures con granada. Las interacciones proteínas-polifenoles dependen de la naturaleza del compuesto fenólico. Las antocianinas, principales

compuestos fenólicos en zumo procedente de arilos, permanecen mayoritariamente con las proteínas. Durante la fermentación y almacenamiento en refrigeración las antocianinas individuales se degradan sin afectar al color de los yogures ni a su elevada capacidad antioxidante. El yogur así desarrollado es un producto bajo en grasa, rico en proteínas lácteas y en compuestos antioxidantes procedentes del zumo de granada. Consideramos que merece más investigaciones sobre sus propiedades y variables tecnológicas del proceso.

Respecto al desarrollo de productos con potencial anti-obesidad, el ácido linoleico conjugado (CLA) presente de forma natural en la leche aumenta a causa del proceso de fermentación. En este estudio observamos que la adición de ácido linoleico libre a la leche, previo a la fermentación potencia este incremento de CLA. La leche de cabra solo presenta el isómero *cis*-9, *trans*-11-CLA, con la adición de ácido linoleico a la leche las bacterias sintetizan además el isómero *trans*-10, *cis*-12-CLA.

En esta Tesis Doctoral se han puesto a punto técnicas de incorporación de extractos de frutas a la elaboración de leches fermentadas y se han comprobado su viabilidad tecnológica, aceptación sensorial y capacidad antioxidante. Se ha conseguido potenciar el contenido de CLA en leche de cabra mediante la fermentación y adición de precursores.

ABSTRACT

The development of fermented milks with health claims requires scientific basis and technological feasibility. This paper addresses strategies for the development of fermented milks with antioxidant potential, by incorporation of plant extracts, and fermented milks with anti-obesity potential by enhancing the content of conjugated linoleic acid (CLA).

Fruit extracts used were blanching water of the industrialization of dates and quince. Pomegranate juice was also tested. All fruit materials were local products. The dates and quinces used were not suitable for fresh consumption and thereby enable us to explore their industrial use: blanching water and pastes were obtained.

Blanching water and pastes of the date and quince industries can be used as ingredients in the formulation of functional foods due to their content in natural antioxidants. The addition of blanching water from date and quince to reconstitute milk for yogurt elaboration causes changes in yogurt: Medjoul dates blanching water provided the best technological and sensory results. Blanching water from quince and Confitera dates, although of greater antioxidant potential, reduced microbial populations and sensory evaluations of the yogurts. Using blanching water from Medjoul dates in yogurt is recommended due to its flavor and sugar content.

Regarding pomegranate addition to yogurt, pomegranate juice can be used up to 40% in the formulation of yogurt to obtain a yogurt of high antioxidant capacity. Milk and juice must be pasteurized separately to avoid destabilization of the milk proteins. These yogurts develop lower acidity and obtain high scores in sensory acceptance.

It is a well-known fact that phenolic compounds have a high affinity for proteins, this has led us to investigate the milk protein-polyphenol interactions in yogurts with pomegranate juice. Protein-polyphenol interactions depend on the nature of the phenolic compound. Anthocyanins, the main phenolics in juice from arils, remain largely bind to protein. During fermentation and refrigerated storage individual anthocyanins are degraded without affecting the color of yoghurt or its high antioxidant capacity. We developed a yogurt low in fat, rich in milk proteins and

antioxidant compounds from pomegranate juice, with promising health benefits. Further research is needed on their properties and technological process variables.

Regarding the potential anti-obesity studies, conjugated linoleic acid (CLA) naturally present in milk increases due to milk fermentation. In this study we observed that the addition of free linoleic acid to the milk, prior to fermentation, further enhanced CLA production. Goat's milk contains only *cis*-9, *trans*-11-CLA isomer, with the addition of linoleic acid to milk the *trans*-10, *cis*-12-CLA isomer is also synthesized.

The present Ph.D. dissertation has set techniques for the incorporation of fruit extracts to the preparation of fermented milks and have proven their technological feasibility, sensory acceptance and antioxidant capacity. It has managed to enhance the CLA content of goat milk by fermentation and addition of precursors.

Capítulo 1: Introducción

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1.1. LECHES FERMENTADAS: TENDENCIAS MUNDIALES Y PAUTAS DE CONSUMO

Según un estudio llevado a cabo por el analista de mercado *Euromonitor International* las ventas mundiales de productos lácteos en 2012 alcanzaron los 425 billones de dólares (308 billones de euros), con una expectativa de venta para el 2018 cercana a los 700 billones de dólares (508 billones de euros) (Hudson, 2013). A nivel mundial la fabricación de productos lácteos fermentados representa la segunda industria fermentadora más importante (únicamente superada por la de bebidas alcohólicas) (Khurana and Kanawjia, 2007).

Las tendencias de consumo están cambiando considerablemente, de modo que cada vez más los consumidores demandan alimentos que les proporcionen salud y bienestar (SB). Durante la última década esta demanda ha aumentado en muchas partes del mundo, en parte debido al incremento de los costos de salud, el aumento de la expectativa de vida y el deseo de una mejor calidad de vida. Esto ha forzado a los consumidores a buscar medios más baratos y efectivos en la protección de su salud (Ozen y col., 2012).

El creciente interés de los consumidores por el papel de la nutrición sobre la salud y el bienestar es el principal factor detrás del éxito del mercado de los SB-productos lácteos. Otro factor importante es el creciente deseo de los consumidores en tener un papel más activo en la optimización de su salud y bienestar personal, sin depender de los productos farmacéuticos (Özer y Kirmaci, 2010). La pregunta entonces es, ¿cómo satisfacer la demanda de 'salud y bienestar' a través del canal de los productos lácteos? La respuesta de la Industria Láctea actual a esta pregunta viene ilustrada en la **Figura 1.**

Esta clasificación representa de manera esquemática el modo en el que los atributos de salud y bienestar se asocian a los productos lácteos así como qué condicionantes de salud llevan al consumidor final a la selección de un producto lácteo u otro en el momento de compra. En la base de la pirámide se encuentran los productos lácteos funcionales y/o fortificados, de ellos se hablará más en profundidad en la siguiente

sección; ejemplos de ellos son los las leches fermentadas pre/probióticas, formulas infantiles enriquecidas, etc. A continuación se encuentran los productos 'naturalmente saludables', este segmento engloba a alternativas para la leche: 'leche' de almendra y licuados de soja, arroz, avena y nueces. A nivel mundial este segmento es el que más ha crecido durante los últimos años (un 32.8% en la temporada 2011-2012), hecho que principalmente se debe a un cambio en el enfoque; el paso de la distribución y venta de estas bebidas sustitutivas en canales especializados (tiendas de dietética, herbolarios) a pequeños y grandes supermercados. Los llamados 'mejor para ti' engloban aquellos dirigidos a la gestión del peso corporal: bajos/cero grasas y/o azúcares. En este segmento la tendencia mundial actual es el uso de ingredientes naturales, como proteínas o la stevia. Ejemplos de bajos/cero grasas son los yogures 0% materia grasa y yogures que contienen el doble de proteínas que los yogures regulares que crean sensación de saciedad. En el terreno de los 'sin azúcar' el protagonismo actual está acaparado por la stevia, un edulcorante natural 300 veces más dulce que el azúcar y sin calorías que se extrae de la planta *Stevia rebaudiana* y en Europa se reguló para su uso alimentario a finales de 2011. En el caso de los productos lácteos orgánicos/ecológicos, los postres lácteos refrigerados y la leche ecológicos son las categorías que más han crecido mundialmente (un 21.7% y 14.6%, respectivamente); el consumo de este segmento de productos está relacionado con el estilo de vida y respeto por el medio ambiente. Por último los productos lácteos para personas afectadas por intolerancias alimentarias, como ejemplo la leche sin lactosa. Aunque en un principio estos productos estaban dirigidos a personas que padecen intolerancias su consumo se está generalizando debido a que empiezan a ser percibidos como productos más digestivos que sus homólogos "con" (Moreno, 2012; Durán, 2013; Hudson, 2013).

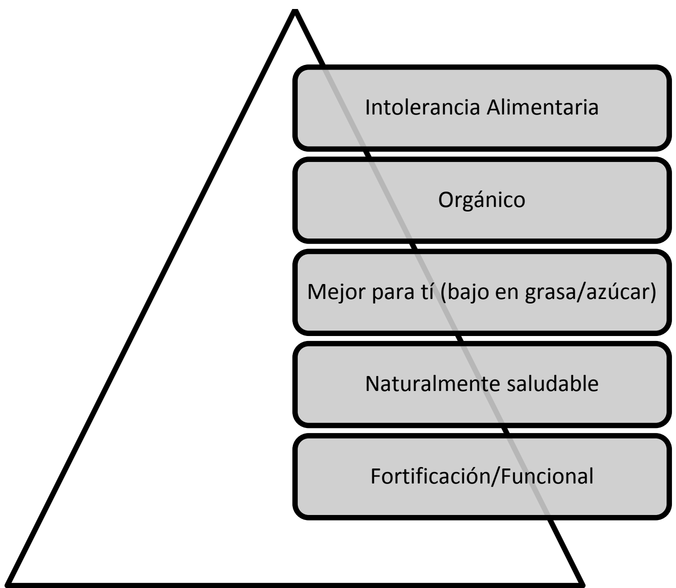

Figura 1. Taxonomía de los productos lácteos en relación a los atributos de salud y bienestar. Adaptado de Hudson (2013).

Los principales contribuyentes al crecimiento de los SB-productos lácteos son las fórmulas de leche líquida funcionales/fortificadas seguidas de los yogures pro/prebióticos, la leche baja en grasa, las bebidas lácteas aromatizadas y fortificadas/funcionales (Hudson, 2013). Las fórmulas infantiles, dentro de la categoría de alimentos y bebidas funcionales, son las que alcanzan un mayor crecimiento en el año 2013 con un volumen de venta mundial de 5 billones de dólares (3.63 billones de euros), seguida por las bebidas energéticas (3.5 billones de dólares, 2.54 billones de euros) y los yogures pre/probióticos (2.4 billones de dólares, 1.74 billones euros) según datos de *Euromonitor International* (Starling, 2013).

Si bien esta tendencia de 'salud y bienestar' no sigue el mismo comportamiento en todas las partes del mundo. Como podemos observar en la **Figura 2** actualmente Europa Occidental es la mayor potencia en ventas, tanto de productos lácteos totales como de los llamados 'sanos y saludables'; los analistas del sector apuntan a que a la vista de unos años el crecimiento en ventas en la región de Asia-Pacífico será considerable pasando a ser la principal potencia.

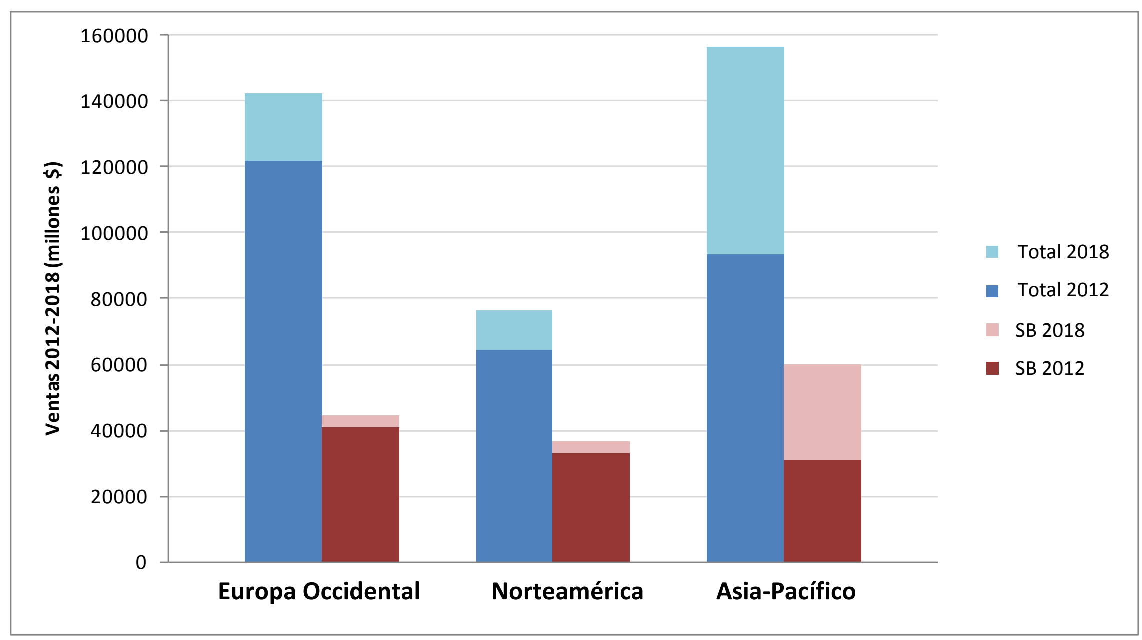

Figura 2. Ventas mundiales de productos lácteos: últimos datos y previsiones para 2018: SB, productos lácteos que proporcionan salud y bienestar; Total, total de productos lácteos. Adaptado de Hudson (2013).

Recientemente la IDF (*International Dairy Federation*) ha publicado los datos de consumo de productos lácteos a nivel mundial (IDF, 2013); en la **Tabla 1** se muestra un breve resumen de los resultados. De los datos publicados queda claro que en general el consumo de productos lácteos a nivel mundial ha aumentado durante el periodo 2006-2012, aunque de manera sostenida. Sin embargo la media de Europa, Norte América, Australia y Nueva Zelanda ha sufrido un ligero retroceso debido principalmente al aumento en sus exportaciones. En el año 2012 Europa ha aumentado un 5% las exportaciones de leche desnatada en polvo, un 12% las de mantequilla, un 14% las de queso y un 10% las de suero (IDF, 2013).

Tabla 1. Consumo aparente per capita de productos lácteos (kg-en equivalentes de leche).

País	2006	2012	%
Mundo	101	108	+7
Asia	61	71	+16
África	42	48	+14
Latinoamérica	129	151	+17
Rusia + Ucrania + Bielorrusia	253	255	+1
Europa + Norteamérica + Australia + Nueva Zelanda	290	284	-2

Fuente: IDF (2013).

1.2. ALIMENTOS FUNCIONALES: DEFINICIÓN Y PATRONES DE CONSUMO

El progreso científico en la comprensión de la relación entre nutrición y salud tiene un profundo impacto en el enfoque de los consumidores sobre la nutrición, lo cual ha resultado en el desarrollo del concepto de "alimento funcional" (Bhat y Bhat, 2011a). Sin embargo, en la actualidad no hay una definición universal aceptada para los alimentos funcionales, que son, tal vez, vistos más precisamente como un concepto que como un grupo bien definido de productos alimenticios (Ozen y col., 2012). La Acción Concertada de la Comisión Europea sobre Ciencias de los Alimentos Funcionales en Europa (Functional Food Science in Europe, FuFoSE) describió los alimentos funcionales como "un alimento puede ser considerado como "funcional" si se demuestra satisfactoriamente que ejerce un efecto beneficioso sobre una o más funciones selectivas del organismo, además de sus efectos nutritivos intrínsecos, de una manera relevante para mejorar el estado de salud y bienestar, reducir el riesgo de enfermedad, o ambas cosas. Los alimentos funcionales deben seguir siendo alimentos y deben demostrar sus efectos en las cantidades en que normalmente se consumen en la dieta: no se trata de comprimidos o cápsulas, sino de alimentos que forman parte de un régimen normal" (Diplock y col., 1999).

Un alimento funcional puede entonces ser un alimento natural o uno que ha sido modificado para tener una influencia funcional sobre la salud y el bienestar del consumidor a través de la adición, eliminación o modificación de componentes específicos (Ozen y col., 2012).

Los alimentos funcionales comprenden alimentos convencionales que contienen compuestos bioactivos de forma natural (ej., fibra dietética) o alimentos enriquecidos con compuestos bioactivos (ej., probióticos, antioxidantes) o ingredientes alimentarios que se incorporan a alimentos tradicionales (ej., prebióticos). Entre los componentes funcionales más comunes se encuentran: probióticos y prebióticos, fibra soluble, ácidos grasos poliinsaturados omega-3, ácido linoleico conjugado, antioxidantes de plantas, vitaminas y minerales, ciertas proteínas, péptidos y aminoácidos, así como fosfolípidos (Bhat y Bhat, 2011b).

En 2005 el mercado mundial de los alimentos funcionales tuvo un valor de 16 billones de dólares (11.62 billones de euros) (Leatherhead Food International, 2006). En España, los alimentos funcionales mueven un volumen de negocio de 2.900 millones de euros anualmente, tienen una cuota del 7% en el mercado de gran consumo, donde crecen a un ritmo del 2% (Resa, 2012). Entre los alimentos funcionales, los productos lácteos funcionales representan casi el 43% del mercado, el cual está casi en su totalidad compuesto por productos lácteos fermentados (Özer y Kirmaci, 2010). En general no se puede establecer una conexión entre la elección de los consumidores respecto a los diferentes alimentos funcionales. El género, la edad, el nivel de educación y el estado de salud personal pueden predecir, cada uno de ellos por separado, el consumo de uno o más alimentos funcionales; y la comprensión de los mensajes saludables de los alimentos funcionales juega un rol esencial en ayudar a guiar la elección de los alimentos (Ozen y col., 2012).

En la presente tesis entenderemos por alimentos funcionales a aquellos que ofrecen mejoras nutricionales potenciales respecto a su homólogo estándar. En este sentido puede tratarse de desarrollos funcionales o de productos en los que se ha reducido o potenciado un ingrediente. Cabe hacer esta aclaración pues dentro del sector lácteo, y como se ha mencionado anteriormente, la tendencia actual mundial es la de denominar a esta categoría como 'sanos y saludables' pues además de lo estrictamente funcional se engloban otras categorías como los ecológicos/orgánicos, los productos lácteos para personas afectadas por intolerancias alimentarias y los productos lácteos "sin" (grasa/azúcares).

1.3. LECHE Y PRODUCTOS LÁCTEOS COMO ALIMENTOS FUNCIONALES

La leche es una mezcla compleja de proteínas específicas, lípidos y sacáridos; contiene numerosas sustancias biológicamente activas como inmunoglobulinas, enzimas, péptidos antimicrobianos, oligosacáridos, hormonas, citoquinas y factores de crecimiento (Clare y Swaisgood, 2000; Donovan, 2006; Pouliot y Gauthier, 2006). Los beneficios de la leche y los productos lácteos son conocidos por la humanidad desde tiempos históricos y son atribuidos a los componentes biológicamente activos que están presentes en la leche y además por su idoneidad en la modulación de actividades a través de la acción de las bacterias probióticas en los productos lácteos fermentados (Bhat y Bhat, 2011b).

Proteínas funcionales, péptidos bioactivos, ácidos grasos esenciales, calcio, vitamina D y otros componentes de la leche poseen efectos positivos sobre los sistemas inmune y cardiovascular, así como el tracto gastrointestinal y la salud intestinal (Rogelj, 2000; Korhonen y Pihlanto, 2006). En la **Tabla 2** se resumen las principales funciones que ejercen los componentes funcionales de la leche (y productos lácteos) así como su contribución a la prevención de diversas enfermedades.

El papel funcional de los productos lácteos fermentados viene dado directamente a través de la interacción con microorganismos consumidos (efecto probiótico) o indirectamente como resultado de la acción de los metabolitos microbianos tales como vitaminas, proteínas, péptidos, oligosacáridos y ácidos orgánicos, generados durante el proceso de fermentación (Bhat y Bhat, 2011b).

Finalmente, la regulación dietética de la ingesta de alimentos mediante productos lácteos tiene potencial para contribuir a la prevención y manejo de la pandemia de la obesidad. El control del peso corporal es la segunda preocupación sobre la salud a nivel mundial y una de las industrias que más dinero mueve, sus ventas mundiales en 2013 fueron de 151 billones de dólares (110 billones de euros), por delante de las ventas dedicadas a la salud intestinal (75 billones de dólares, 55 billones de euros) y únicamente superadas por las orientadas al bienestar general (437 billones de dólares, 317 billones de euros) (Hudson, 2013).

Tabla 2. Leche y productos lácteos: principales funcionalidades.

Componente		Actividad biológica	Referencia
Proteínas	Caseínas	Precursor de péptidos biológicamente activos que, entre otras funciones, actúan como antihipertensivos.	Gobbetti y col., (2004).
		Caseín fosfopéptidos (CPP): son portadores de cationes en el intestino. Inhiben la formación de caries.	Oukhatar y col., (2002).
	β-Lactoglobulina	Immunomodulador. Debido a que la molécula contiene una parte hidrofóbica puede unir vit A, vit D, calcio y ácidos grasos.	Brown (1984); Wang y col., (1997); Beaulieu y col., (2006).
		Péptidos derivados: propiedades antihipertensivas, antitrombóticas, opioide, antimicrobianas, immunomodulantes, hipocolesterolémicas y actividad secuestrante de radicales.	Hernández-Ledesma y col., (2007).
	α-Lactoalbúmina	Actividad anticancerígena. Propiedades antiulcerativas.	Svensson y col., (2000); Mezzaroba y col., (2006).
		Péptidos derivados: efectos immunomodulatorios y actividad antimicrobiana.	Jaziri y col., (1992); Pellegrini, (2003).
	Immunoglobulinas	Prevención de infecciones gastrointestinales. Protección frente patógenos microbianos. Prevención de caries dental. Disminución del colesterol.	Playne y col., (2003).
	Lactoferrina	Regulación de la homeostasis del hierro. Defensa frente un amplio rango de infecciones microbianas. Actividad anti-inflamatoria. Protección frente al cáncer.	Wakabayashi y col., (2006); Hartmann y Meisel, (2007).

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		Péptido derivado: lactoferricina. Responsable de sus efectos immunomodulatorios y antimicrobianos.	
Ácidos grasos	Ácido butírico (C4:0)	Importante anticarcinógeno. Junto a los lípidos etéricos, vit A, D, E y CLA forman una barrera protectora frente a diferentes enfermedades no transmisibles.	Parodi (1999).
	Ácidos caprílico y cáprico (C8:0 y C10:0)	Actividad antivírica.	Thormar y col., (1994)
	Ácido láurico (C12:0)	Funciones antivíricas y antibacterianas. Actividad anti-caries y anti-placa.	Sun y col., (2002a); Thormar y Hilmarsson, (2007).
Ácido linoleico conjugado (CLA)		Actividad anti-carcinogénica. Actividad anti-obesidad Prevención aterosclerosis. Prevención en la modulación de ciertos aspectos del sistema inmune.	Smedman y Vessby, (2001); Park y Pariza, (2007); Dilzer y Park, (2012).
Esfingolípidos		Inhibición de cáncer. Actividad antimicrobiana e inmunomoduladora. Inhibición de la absorción de colesterol.	Akalin y col., (2006).
Calcio		Prevención de osteoporosis. Promoción para huesos y dientes sanos. Protección frente hipertensión.	Playne y col., (2003).
Galacto-oligosacáridos		Aumento del crecimiento de bacterias beneficiosas en el intestino. Mejora de la absorción de calcio. Reducción de los niveles de triglicéridos y colesterol. Protección frente infecciones en bebés.	Playne y col., (2003).
Bacterias ácido lácticas		Promoción de la salud intestinal. Reducción de los efectos de una infección intestinal. Aumento de la actividad inmune. Reducción de eczemas atópicos en bebés.	Playne y col., (2003).

1.4. INGREDIENTES BIOACTIVOS EN LA FORMULACIÓN DE PRODUCTOS LÁCTEOS FUNCIONALES

En los últimos años se han lanzado al mercado un gran número de bebidas lácteas funcionales que contienen un amplio rango de compuestos bioactivos (no probióticos), principalmente en Estados Unidos, Europa y Japón. Las bebidas lácteas no fermentadas y los yogures batidos son los productos lácteos más usados como vehículos de compuestos bioactivos (**Tabla 3**).

Tabla 3. Algunos ejemplos de bebidas lácteas funcionales comerciales con ingredientes bioactivos.

Producto	Marca	Fabricante	Fuente de bioactividad	Comentarios
Leche desnatada (fresca)	Dawn Omega Milk	Dawn Dairy Ireland	AG omega-3 (provenientes de aceite de pescado)	Fue la primera leche con omega-3 pasterizada en Europa.
Leche fresca y chocolateada	Dairy Oh	Neilson (Canadá)	AG omega-3	Proporciona hasta 20 mg de DHA por ración. Además incluye vit A y D.
Bebida láctea fermentada con fruta	Evolus®	Valio Finland	Péptidos bioactivos obtenidos tras fermentación (Val-Pro-Pro; Ile-Pro-Pro).	Evolus® reivindica que es el primer alimento funcional Europeo que disminuye la presión arterial.
Leche rica en CPP e IgG	Alpha	Stolle Milk Japan	Enriquecida en CPP e IgG	
Leche desnatada	Flora Pro-Activ	Unilever (Reino Unido)	Fitosteroles	Reivindica que 'está demostrado clínicamente que reduce el colesterol'
Bebida láctea desnatada	Danacol	Danone	Fitosteroles	1.6 g fitosteroles por cada 100 mL. Reclama que 'está demostrado que el consumo de 1.6 g de esteroles de plantas al día, como parte de una dieta saludable, reduce el colesterol'.
Yogur líquido	Benecol	Mc Neil Nutritionals (Finlandia)	Fitoesteroles	Tecnología licenciada de Raiso, Finlandia. Contiene un 3% de esteres de fitoesteroles.
Bebida a base de leche y fruta	Naturlinea[1]	Corporación Alimentaria Peñasanta S.A.	CLA	Reivindica que "ayuda a reducir la grasa".

		(España)		
Leche	Natrel Calcium	Natrel (Canadá)	Calcio	Contiene un 35% más de calcio que la leche corriente.
Leche	Meiji Love	Meiji Milk (Japón)	Calcio y hierro	Contiene 350 mg de calcio y 3 mg de hierro por cada 200 mL.
Leche	Magnesio	Lactalis (Francia)	Magnesio	

Abreviaturas: AG, ácidos grasos; CPP, caseín fosfopéptidos; CLA, ácido linoleico conjugado.
[1]Actualmente ya no se comercializiza.
Adaptada de Sharma (2005) y Özer y Kirmaci (2010).

1.4.1. *Ácidos grasos omega-3 y omega-6*

La composición de la grasa de los productos lácteos puede ser alterada mediante la reducción de la ratio de ácidos grasos saturados/insaturados de forma que se vea elevado el contenido en ácidos grasos que sean más deseables para la nutrición humana, tales como los ácidos grasos poliinsaturados (AGPI) (Khurana y Kanawjia, 2007). Los AGPI se dividen en dos grandes familias: la serie omega-6, o n-6, derivada del ácido graso linoleico (LA, 18:2n-6) y la serie omega-3, o n-3, derivada del ácido α-linolénico (ALA, 18:3n-3). Tanto LA como ALA son considerados ácidos grasos esenciales ya que los mamíferos, incluyendo los humanos, carecen de las enzimas necesarias para su síntesis. Por tanto, estos ácidos grasos sólo pueden ser obtenidos a través de la dieta (Das, 2006). La importancia del ALA es ampliamente conocida y es debida a que es el precursor de otros ácidos grasos de cadena larga que no pueden ser sintetizados en el cuerpo humano: el ácido eicosapentaenoico (EPA, 20:5n-3) y el ácido docosohexaenoico (DHA, 22:6n-3). Los ácidos grasos EPA y DHA están presentes de forma natural en los aceites de pescado y en algas marinas, ALA en las semillas de lino y varios aceites vegetales y nueces y LA en aceites de cártamo, girasol, sésamo, soja y maíz (Shireman, 2003). Los ácidos EPA y DHA son esenciales para el desarrollo del cerebro, concentración, y la capacidad de aprendizaje en los niños (Milner y Alison, 1999); además en base a la evidencia de numerosos estudios los AGPI n-6 y n-3 son considerados beneficiosos para la prevención de enfermedades cardiovasculares (ECV) (Lee y col., 2008), prevención de ciertos tipos de cáncer (Daniel y col., 2009; Thiébaut y col., 2009) y la mejora en el metabolismo lipídico y prevención de la obesidad y

diabetes (Flachs y col., 2009), entre otras. El aumento de los niveles de estos ácidos grasos saludables en los productos lácteos puede conseguirse mediante el uso de ciertas bacterias lácticas durante la fermentación de la leche (Martín-Diana y col., 2004) o mediante la sustitución de la grasa láctea por aceites con elevados niveles de AGPI (Luna y col., 2004). Otra posibilidad para el aumento de los niveles de los AGPI n-3 en la leche sería incluir aceites de vegetales, de pescado o algas marinas en la dieta de los animales (Dave y col., 2002; Kolanowski y col., 1999).

El desarrollo de productos enriquecidos en AGPI supone un desafío para la industria alimentaria, tanto desde el punto de vista de la oxidación lipídica como de la aceptación sensorial por parte del consumidor (Sharma, 2005). Además en ciertos estudios la textura del producto final se vio afectada (en yogures: falta de firmeza y mayor sinéresis en yogures) aunque el uso de concentrados de proteínas puede dar solución a este problema (Dave y col., 2002; Martín-Diana, 2004).

1.4.2. Proteínas y péptidos alimentarios

Como ya se ha comentado en apartados anteriores (sección 1.3) es un hecho establecido que las proteínas alimentarias, especialmente las proteínas lácteas, pueden actuar como precursores de péptidos biológicamente activos con diferentes funciones y efectos fisiológicos, como por ejemplo su actividad anti-hipertensiva. Los caseín fosfopéptidos (CPP) son péptidos derivados de la caseína que contienen residuos de fosfato en forma de serin monoester que forman sales organofosfatadas. Debido a su elevada carga negativa en la estructura resultante de la fosforilación, las CPPs pueden unir macroelementos como el calcio, magnesio y hierro así como un amplio rango de microelementos como zinc, bario, selenio, cobalto, cromo y níquel en el intestino (Oukhatar y col., 2002). Sin embargo, aunque el papel funcional y metabólico de las CPPs está bien documentado y no existen riesgos para la salud asociados con su consumo, actualmente no existen leches enriquecidas en CPP en el mercado (Özer y Kirmaci, 2010).

1.4.3. Fitoesteroles y fitoestanoles

Los fitoesteroles son un grupo de esteroles derivados de las plantas con una estructura similar a la del colesterol, por lo que son capaces de interferir con la absorción de éste en el tracto intestinal siendo una forma natural de conseguir un nivel bajo de colesterol en la sangre (Heinemann y col., 1991). En base a recientes publicaciones los fitoesteroles han mostrado tener potencial frente a la inhibición de canceres de estómago, hígado, ovarios y mamas (Woyengo y col., 2009). Cuando se adicionan a alimentos, los fitoestanoles reducen de forma efectiva la absorción de los esteroles del tracto digestivo por lo que también contribuyen a la disminución de los niveles de colesterol (Khurana y Kanawjia, 2007). Jones y col., (1999) demostraron que la inclusión de 1.7 g al día de fitoesteroles en la dieta de hombres hipercolesterolémicos tenía el efecto de disminuir el colesterol sanguíneo. En un estudio más reciente se comprobó que el consumo diario de una leche desnatada que contenía 1.6 g de fitoesteroles redujo en un 8% los niveles de la lipoproteína de baja densidad (LDL) tras 6 semanas (Hansel y col., 2007). La Autoridad Europea de Seguridad Alimentaria (EFSA) y la Estadounidense *Food and Drug Administration* (FDA) han aceptado los esteroles de plantas como ingredientes alimentarios (EFSA 2009; FDA 2013).

Los problemas tecnológicos asociados con la incorporación de los fitoesteroles a los productos lácteos están relacionados con la pobre solubilidad que presentan en agua, lo cual los hace especialmente difícil de incorporar en productos lácteos bajos en grasa (Sharma, 2005).

1.4.4. Isoflavonas

Las isoflavonas, también llamadas "fitoestrógenos", son una amplia y distintiva subclase de flavonoides. Son estructural y funcionalmente similares al estradiol (hormona femenina) pero mucho menos potentes. Debido a esta similaridad las isoflavonas han probado tener efectos preventivos frente a muchos tipos de enfermedades de tipo hormonales (Uzzan y Labuza, 2004). Las isoflavonas están mucho más limitadas al reino vegetal que otros flavonoides ya que se encuentran casi exclusivamente en legumbres, especialmente en la soja (Puupponen-Pimiä y col., 2002). Las isoflavonas poseen propiedades antioxidantes, comparables incluso a las de la vitamina E. El poder antioxidante de las isoflavonas puede reducir el riesgo a largo

plazo de padecer cáncer mediante la prevención del daño de radicales libres al ADN. La genisteína es el antioxidante más potente entre las isoflavonas de soja seguido de la daidzeína. En la naturaleza las isoflavonas se presentan como glucósidos y una vez desconjugados por la flora intestinal pueden ser absorbidos a la sangre (Awaisheh y col., 2005).

Desde un punto de vista tecnológico, la mayoría de las isoflavonas presentan pobre solubilidad en agua y pueden dar lugar a defectos de flavor como amargor y sabor a legumbre (Özer y Kirmaci, 2010).

1.4.5. Ácido linoleico conjugado

En la actualidad, los ácidos grasos conjugados han atraído considerablemente la atención debido a sus potenciales efectos beneficiosos sobre la salud. Ácido linoleico conjugado (CLA) es el término colectivo utilizado para un grupo de isómeros posicionales (C8,C10; C9,C11; C10,C12; y C11,C13) y geométricos (cis,cis; cis,trans; trans,cis; y trans,trans) del ácido linoleico con un sistema de dobles enlaces conjugados (Moon y col., 2008). El CLA se encuentra de forma natural casi en exclusiva en productos de origen animal. El CLA ha demostrado tener propiedades anti-cancerígenas, anti-obesidad y anti-diabéticas (Nagao y Yanagita, 2005). Debido a que los productos lácteos con alto contenido en CLA tienen un tremendo potencial dentro del mercado de los nutraceuticos y funcionales el interés en la investigación con CLA ha crecido considerablemente. El yogur con CLA es el producto lácteo más accesible en el mercado (Özer y Kirmaci, 2010).

En la revisión realizada por Bisig y col., (2007) se informa de que las etapas de calentamiento y fermentación no tienen efectos negativos sobre el contenido del CLA, excepto el calentamiento por microondas que causó una disminución del 53% de CLA en leche.

1.4.6. Minerales y vitaminas

Vitaminas y minerales suelen ser adicionados a bebidas lácteas fermentadas y no fermentadas para compensar sus pérdidas durante el procesado. Calcio, magnesio y hierro son los minerales más comúnmente añadidos.

El **calcio** es el quinto elemento más abundante en el cuerpo humano y contribuye de forma significativa a la densidad mineral ósea. Una ingesta adecuada de calcio es crítica durante todas las etapas de la vida. Es particularmente importante para el desarrollo óseo durante la adolescencia y para la prevención de la pérdida ósea (osteoporosis) en mujeres postmenopáusicas (Wallace y col., 2013). Recientemente se ha demostrado que la fortificación de un producto lácteo (yogur) con calcio y vitamina D_3 proporciona una mayor prevención en la resorción ósea en comparación que su equivalente no fortificado (Bonjour y col., 2013). En este sentido los productos lácteos son considerados como fuente de calcio, 200 mL de leche (ración típica) proporcionan cerca del 22% de la cantidad diaria recomendada (RDA) (Cashman, 2006). El calcio puede adicionarse a la leche y bebidas lácteas en forma de sales inorgánicas como cloruro de calcio, lactato de calcio, gluconato de calcio o en forma de calcio de base láctea. Las sales de calcio pueden causar una bajada de pH y por lo tanto alterar la estabilidad térmica de la leche durante el procesado; para dar solución al problema se suele ajustar el pH original con fosfato de sodio (Singh y col., 2007). El mayor desafío para la industria es la pobre solubilidad de las sales de calcio que llevan a la formación de sedimentos en el producto como resultado de inestabilidad proteica inducida por el calcio (Sharma, 2005).

El **hierro** es un oligoelemento esencial en la nutrición humana; su deficiencia induce anemia (la cual afecta al 30% de la población mundial), alteración en el desarrollo mental y un descenso en la inmunidad. La fortificación de leche y productos lácteos con hierro es una buena estrategia para prevenir estos desórdenes. Sin embargo, una fortificación directa provocaría varias modificaciones biofisicoquímicas con importantes consecuencias como cambios en flavor y oxidación lipídica, descenso de pH y modificación de las caseínas, entre otros (Gaucheron, 2000). Uno de los criterios más importantes a tener en cuenta en la selección del alimento a ser fortificado es la interacción entre micronutrientes. La absorción del hierro está positivamente correlacionada con las vitaminas A, C, E y ácido fólico; y negativamente con calcio, fósforo, magnesio, malonaldehido, polifenoles y ácido oxálico y fítico (Martínez-Navarrete y col., 2002). Además es probable que la absorción del hierro se vea

aumentada por la fermentación debido a la producción y acumulación de ácido láctico y otros ácidos orgánicos (Branca y Rossi; Silva y col., 2008).

Otros minerales como el **magnesio** y **selenio** son también buenos candidatos para la elaboración de bebidas lácteas funcionales. El nivel de Mg en leche es tal que 200 mL proporcionarían alrededor del 6% de la RDA. Una deficiencia en Mg podría estar relacionada como factor de riesgo de padecer osteoporosis (Cashman, 2006) y diferentes tipos de depresión (Serefko, 2013). Además niveles bajos de Mg están asociados con un gran número de enfermedades crónicas, tales como migrañas, Alzheimer, infartos, hipertensión, enfermedades cardiovasculares y diabetes tipo 2 (Volpe, 2013). Aunque sí existen referencias comerciales de bebidas lácteas enriquecidas en Mg, no las hay de selenio y esto es debido principalmente a que se pierde durante el proceso de pasterización. El selenio es uno de los elementos que determinan el normal funcionamiento del organismo; presenta propiedades antioxidantes y protege al organismo frente a la acción de radicales libres y factores carcinogénicos (Kieliszek y Błażejak, 2013).

En cuanto a **vitaminas**, la leche no está considerada como fuente principal de ellas aunque es relativamente rica en algunas del grupo B. Sin embargo, durante el procesado de la leche alguna de las vitaminas puede sufrir degradación variando sus niveles. Por ello varios estudios se han llevado a cabo para comprobar la estabilidad de las vitaminas durante la elaboración de productos lácteos. Por ejemplo, Ottaway (2009) comprobó que el nivel de pérdidas de ácido fólico en leche pasterizada, esterilizada y UHT fue de 5, 30 y 20% respectivamente. Papastoyiannidis y col., (2006) fortificaron cuatro tipos de leches fermentadas con varias vitaminas del grupo B; ellos comprobaron que a pesar de las pérdidas sufridas por el tratamiento térmico y la fermentación el nivel de las vitaminas permaneció estable durante 16 días de almacenamiento. Actualmente es habitual el enriquecimiento de leches fermentadas con vitamina B6 para acogerse a las alegaciones nutricionales previstas en el Reglamento (UE) nº 432/2012 (por el que se establece una lista de declaraciones autorizadas de propiedades saludables de los alimentos).

1.4.7. Fibra dietética

En el mercado de los alimentos funcionales los productos focalizados para la salud intestinal han liderado el campo de la investigación y el desarrollo. Cuando se plantea el diseño de alimentos que promuevan la salud a través de reacciones con la microbiota intestinal se usan tres tipos de ingredientes alimentarios: microorganismos vivos (probióticos), carbohidratos no digestibles (fibra dietética y prebióticos) y metabolitos bioactivos secundarios como los polifenoles (Puupponen-Piamiä y col., 2003). En este sentido los probióticos que contienen fruta son los predilectos para los consumidores (Espírito Santo y col., 2011). La fibra dietética contiene todas las características requeridas para ser considerada como ingrediente en la formulación de alimentos funcionales debido a sus efectos beneficiosos sobre la salud. Entre los más destacados están: efecto laxante, reducción del colesterol sanguíneo, reducción de la glucosa en sangre y reducción del riesgo de trastornos crónicos, como enfermedad coronaria, diabetes, obesidad y algunas formas de cáncer (Elluch y col., 2011).

Como ejemplos se han desarrollado leches fermentadas con fibra de cítricos (Sendra y col., 2008); yogures fortificados con fibra de trigo, bambú, inulina y manzana (Staffolo y col., 2004); yogur con fibra de dátil (Hashim y col., 2009), todos ellos con una gran aceptación sensorial. Por otro lado en productos con un mayor contenido en grasa (helados y yogures helados) la adición de fibras como alginatos, goma guar y geles de celulosa no sólo sustituye a la grasa si no que proporciona viscosidad, mejora la emulsión, espumado y la estabilidad a la congelación/descongelación, entre otros (Alexander, 1997).

Los principales problemas tecnológicos en la formulación de un producto lácteo con fibra son la pobre suspensión y la sedimentación (Sharma, 2005).

1.5. Uso de extractos vegetales como fuente de compuestos fenólicos para el enriquecimiento de productos lácteos

Es un hecho establecido que el consumo de frutas y verduras ejerce un efecto beneficioso sobre la salud; esto se debe principalmente a que son ricos en compuestos tales como fibras, folatos, antioxidantes, vitaminas y a un gran número de fitoquímicos

no nutrientes como los compuestos fenólicos (Chong et al., 2010). Como a continuación se explicará numerosos estudios apoyan la contribución de los compuestos fenólicos en la prevención de enfermedades crónicas degenerativas como el cáncer, aterosclerosis y enfermedades cardiovasculares, desórdenes en el sistema nervioso central así como el envejecimiento. Los efectos beneficiosos de los compuestos fenólicos han sido atribuidos a su actividad antioxidante (Heim et al., 2002).

1.5.1. Clases de polifenoles

Todos los compuestos fenólicos surgen de un intermediario común, la fenilalanina, o de un precursor cercano, el ácido siquímico. Estos provienen biogenéticamente de dos rutas sintéticas: la ruta del siquimato y la ruta del acetato (Herrman, 1995). Los compuestos fenólicos pueden clasificarse en diferentes grupos en función del número de anillos de fenol que contengan y de los elementos estructurales que unan esos anillos. Básicamente los principales grupos incluyen: ácidos fenólicos (que a su vez se dividen en dos grandes subgrupos, ácidos hidroxibenzoicos y ácidos hidroxicinámicos), flavonoides, estilbenos y lignanos (Pandey y Rizvi, 2009) (**Tabla 4**).

Estructuralmente, los compuestos fenólicos comprenden un anillo aromático unido a uno o más grupo hidroxilo. Su estructura puede ser desde una molécula fenólica simple hasta un polímero complejo de alto peso molecular. Los flavonoides, los cuales presentan una estructura tipo C_6-C_3-C_6, representan más de la mitad de los 8000 compuestos fenólicos diferentes que existen (Balasundram et al., 2006; Bravo, 1998). En la **Tabla 5** se ilustran las diferentes sub-clases de flavonoides así como su estructura y ejemplos.

En cuanto a su biodisponibilidad, es un tema que hoy en día está en discusión y depende de factores asociados con el procesado del alimento, con el organismo huésped (sexo, edad, composición de la flora intestinal) así como de las interacciones de los polifenoles con otras moléculas como proteínas salivares y enzimas digestivos. La mayoría de los polifenoles presentes en alimentos y bebidas no están disponibles biológicamente ya que se encuentran como polímeros o glicosidos que deben de ser

degradados por las enzimas digestivas o secretados por la microflora colónica (Lewandowska y col., 2013).

Tabla 4. Clases de polifenoles: estructura y ejemplos.

Polifenol	Ejemplo	Fuente dietética	Estructura química
Ácidos fenólicos	Ácido gálico Ác. protocatéquico Ácido cafeico Ácido ferúlico	Arándanos Cereales Aceite de semillas Albaricoques Zanahorias Cítricos	
Flavonoides	Quercetina Miricetina Catequinas Cianidina	Manzanas Uvas Cebollas Soja	
Estilbenos	Resveratrol Piceído Astringin	Uvas Cacahuetes Granada Bayas	
Lignanos	Secoisolariciresinol Matairesinol	Trigo Semillas de lino Semillas de sésamo Cebada	

Fuente: Pandey y Rizvi, (2009).

En general la biodisponibilidad de los polifenoles es más bien baja y además extremadamente variable. Valores de excreción relativa urinaria de su ingesta varían desde un 0.3% para antocianinas hasta un 43% para isoflavonas (Manach y col., 2005). Esta biodisponibilidad puede ser incluso menor cuando el polifenol posee un gran peso molecular, como en el caso de los taninos hidrolizables y condensados y de flavonoides complejos conjugados con varios azúcares y acilados con ácidos hidroxicinámicos. El contenido en los alimentos de estos fenoles complejos es generalmente mayor que los simples; además en muchos artículos científicos estas moléculas complejas han sido subestimadas debido a problemas analíticos (Landete, 2013).

Tabla 5. Clases de flavonoides: estructura y ejemplos.

Flavonoides	Ejemplos	Fuente dietética	Estructura química
Flavonoles	Quercetina Miricetina Kaempferol Isorhamnetin	Cebolla Manzana Arándano rojo Bayas Té Olivas Plátanos Vino tinto	
Flavanonas	Naringenina Hesperetina Eriodictiol	Naranja Limón Piña Pomelo	
Flavanoles	Epicatequín Catequinas Galocatequinas Teoflavina	Lechuga Arándano Uva Té verde Té negro Ciruela Manzana Arándano	
Flavonas	Apigenina Luteolina	Manzana Apio Limón Perejil Orégano Remolacha	
Antocianinas	Cianidina Delfinidina Malvidina Pelargonidina Peonidina Petunidina	Arándano Cereza Uva Frambuesa Fresa Arándano rojo	
Isoflavonas	Daidzeína Genisteína Gliciteína Biochanina A Formononentín	Soja Legumbres	

Fuente: Pandey y Rizvi, 2009.

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1.5.2. Principales fuentes de polifenoles en alimentos

La principal fuente dietética de polifenoles son las frutas, verduras y las bebidas (zumos, vino, té). En 2006 Balasundram y col. realizaron una extensa revisión sobre el

contenido en fenoles en frutas y verduras. Aunque no publicaron datos sobre dátil, membrillo o granada en la **Tabla 6** se presentan los datos de las frutas y verduras más representativos de la zona.

Tabla 6. Contenido en fenoles en varias frutas y verduras.

	Contenido total en fenoles	Fuente
Frutas		
Manzana	296.3 ± 6.4[a]	Sun y col., (2002b)
Plátano	90.4 ± 3.2a	Sun y col., (2002b)
Ciruela negra	143.5 ± 40.6[b]	Karakaya y col., (2001)
Mora	417-555[a]	Sellappan y col., (2002)
Arándano	171-961[a]	Moyer y col., (2002)
Cereza	105.4 ± 27.0[b]	Karakaya y col., (2001)
Arándano rojo	527.2 ± 21.5[a]	Sun y col., (2002b)
Mango	56.0 ± 2.1[a]	Luximon-Ramma y col., (2003)
Melocotón	84.6 ± 0.7[a]	Sun y col., (2002b)
Persimon	1.45[c]	Gorinstein y col., (1999)
Piña	94.3 ± 1.5[a]	Sun y col., (2002b)
Ciruela	174-375[a]	Kim y col., (2003)
Uva roja	220.6 ± 61.2[c]	Karakaya y col., (2001)
Fresa	160 ± 1.2[a]	Sun y col., (2002b)
Verduras		
Brócoli	101.6 ± 1.24[a]	Chu y col., (2002)
Zanahoria	56.4 ± 5.1[a]	Chu y col., (2002)
Pepino	48.0 ± 0.9[b]	Kaur y Kapoor (2002)
Menta	399.8 ± 3.2[b]	Kaur y Kapoor (2002)
Espinacas	91.0 ± 8.5[a]	Chu y col., (2002)
Tomate	25.9-50.0[c]	Martínez-Valverde y col., (2002)
Cebolla	76.3 ±1.9[a]	Chu y col., (2002)

[a] Equivalentes de mg ácido gálico/100 g (peso fresco).
[b] Equivalentes de mg catequina/100 g (peso fresco).
[c] Equivalentes de mg ácido ferúlico/100 g (peso fresco).
Adaptado de Balasundram y col., (2006).

En general, frutas y verduras ricas en antocianinas (p.e., fresas, frambuesa y ciruela roja) han demostrado poseer la mayor actividad antioxidante, seguidas de aquellas ricas en flavanonas (p.e., naranja y pomelo) y flavonoles (p.e., cebolla, puerro, espinacas y col verde), mientras que las frutas ricas en ácidos hidroxicinámicos (p.e., manzanas, tomate, pera y melocotón) presentan las menores actividades antioxidantes (Proteggente y col., 2002).

El contenido en fenoles depende de un número de factores intrínsecos (genero, especie, cultivar) y extrínsecos (agronómicos, ambientales, de manejo y almacenamiento) (Tomás-Barberán y Espín, 2001). Además el procesado y almacenamiento pueden tener diversos impactos sobre su contenido (Häkkinen y col., 2000).

Bebidas como zumos de frutas, té y vino son también una fuente importante de compuestos fenólicos en la dieta. En estudios donde se compara el contenido en fenoles en zumos comerciales frente a zumos frescos se han observado reducciones o pérdidas, las cuales se han atribuido a las técnicas de procesado (Spanos y col., 1990); aunque como se puede comprobar en la **Tabla 7** esto no siempre sucede. En cuanto a la estabilidad de los compuestos fenólicos en zumos se han publicado diferentes resultados. El contenido en fenoles en zumo de manzana demostró ser bastante estable durante un mes de almacenamiento, particularmente a bajas temperaturas (van der Sluis y col., 2005). En el estudio llevado a cabo por Klimczak y col., (2007) el contenido en ácidos hidroxicinámicos se vio bastante afectado tanto por el tiempo como por la temperatura de almacenamiento; en cambio el contenido en flavanonas apenas sufrió cambios demostrando una gran estabilidad. La técnica de procesado empleada también es un factor importante a tener en cuenta. El contenido en fenoles en zumo de granada varió significativamente según la técnica aplicada en el estudio llevado a cabo por Fischer y col., (2011).

Los polifenoles más abundantes en el vino son resveratrol, quercetina, miricetina y catequinas, especialmente en el vino tinto. El café es también una de las mayores fuentes de polifenoles. El mayor polifenol en el café es el ácido clorogénico, un éster de los ácidos cafeico y quínico. Otra fuente importante de compuestos fenólicos es el té; sus propiedades antioxidantes han sido ampliamente estudiadas ya que contienen hasta un 30% de compuestos fenólicos en peso seco. Té verde, té negro y té oolong derivan de las hojas de la planta *Camellia sinensis* y aunque contienen un gran surtido de compuestos los componentes más significativos son las catequinas. De ellas las más importantes son: epigalocatequín-3-galato (EGCG), apigalocatequín (EGC), epicatequín-3-galato (ECG) y epicatequín (EC) (Lin y col., 1998; Pandey y Rizvi, 2009).

Tabla 7. Contenido en fenoles en varios zumos de frutas.

Zumo	Contenido en fenoles totales	Fuente
Comercial		
Granada	186.8-9052.5[a]	Fischer y col., (2011)
Naranja	634.6-684.2[b]	Klimczak y col., (2007)
Manzana	339 ± 43[a]	Gardner y col., (2000)
Pomelo	535 ± 11[a]	Gardner y col., (2000)
Piña	358 ± 3[a]	Gardner y col., (2000)
Fresco		
Granada	1500-4500[a]	Mena y col., (2011)
Arándano	2300 ± 0.4[a]	Jensen y col., (2002)
Naranja	382-1147[c]	Rapisarda y col., (1999)
Uva (roja)	1728[a]	Sánchez-Moreno y col., (1999)
Uva (blanca)	519[a]	Sánchez-Moreno y col., (1999)

[a] Equivalentes de mg ácido gálico/L.
[b] Equivalentes de mg ácido cafeico/L.
[c] Equivalentes de ácido ferúlico/L.

1.5.3. Efectos biológicos de los polifenoles

Un **antioxidante** es una molécula capaz de disminuir o prevenir la oxidación de otra molécula (Halliwell, 1990). Las reacciones de oxidación pueden producir radicales libres los cuales comienzan reacciones en cadena que finalizan dañando células. Los antioxidantes finalizan estas reacciones en cadena eliminando radicales libres intermediarios, y por tanto inhibiendo otras reacciones de oxidación siendo oxidados ellos mismos (Genestra, 2007). Los polifenoles presentes en alimentos pueden ayudar a limitar el daño oxidativo actuando directamente sobre las especies reactivas de oxígeno (ROS) o estimulando sistemas endógenos de defensa (Förstermann, 2008). Las ROS pueden inducir estrés oxidativo y dañar todo tipo de moléculas incluyendo lípidos y proteínas y en último lugar causar patologías como cáncer, enfermedades cardiovasculares, osteoporosis y envejecimiento (Pandey y Rizvi, 2009). Los grupos fenol de los polifenoles pueden aceptar un electrón formando radicales fenoxilo (relativamente estables) y por lo tanto interrumpir la reacción de oxidación en cadena. El fenol por sí mismo es inactivo como antioxidante, pero *orto*- y *para*- difenoles poseen capacidad antioxidante, la cual aumenta con la sustitución de átomos de hidrógeno por grupos etilo o *n*-butilo. Los flavonoides están considerados como uno de los antioxidantes más potentes del reino vegetal debido a que poseen uno o más de los siguientes grupos estructurales: (1) un grupo *o*-difenólico (en el anillo B), (2) un

doble enlace conjugado en la posición 2-3 con la función 4-oxo y (3) grupos hidroxilo en las posiciones 3 y 5. La quercetina combina todas estas características y es considerada uno de los antioxidantes naturales más potentes (Landete, 2013).

La actividad **anti-proliferativa** de los polifenoles frente al desarrollo de cáncer es una de las propiedades más documentadas. Diversos estudios *in vitro* e *in vivo* se han llevado a cabo sobre la investigación de los polifenoles sobre la inhibición de la transformación celular y proliferación. Por ejemplo, Katsube y col., (2003) compararon la actividad anti-proliferativa de 10 extractos de bayas comestibles (en etanol) sobre células humanas leucémicas HL-60 y células humanas HCT-116 (línea celular del carcinoma colorrectal humano), demostrando que el extracto de arándano (bilberry) era el más efectivo. Ross y col., (2007) comprobaron que la actividad anti-proliferativa del extracto de frambuesa sobre las células del cáncer cérvico-uterino (HeLa) estaba predominantemente asociado con los elagitaninos. Otros extractos fenólicos o compuestos fenólicos ampliamente estudiados provienen de olivas, legumbres, cítricos, manzanas y curcumina. Por ejemplo, la genisteína proveniente de la soja puede inhibir el crecimiento de varias líneas de células cancerígenas (leucemia, linfoma, próstata, pecho, pulmón) (Sarkar y Li, 2002). Además de los estudios *in vitro* sobre líneas de células cancerígenas se han llevado a cabo numerosos experimentos *in vivo* con el fin de verificar la eficacia antitumoral (Yang y col., 2002; Gerhauster, 2008; Thomasset y col., 2009). Finalmente numerosos estudios de intervención humana avalan la actividad anti-cancerígena preventiva de los polifenoles. Por ejemplo, Spormann y col., (2008) evaluaron los efectos del consumo de un zumo de frutas rico en antocianinas sobre el estado antioxidante de pacientes en hemodiálisis que presentaban un riesgo elevado de cáncer, arteriosclerosis y otras enfermedades, en parte atribuidas al incremento del estrés oxidativo. Los autores concluyeron que el consumo de zumos de bayas se postula como una medida preventiva para reducir enfermedades crónicas en pacientes expuestos a un aumento en el estrés oxidativo como pacientes en hemodiálisis. Pese a ello, a fecha de hoy, la Organización Mundial de la Salud (OMS) no ha definido una ingesta diaria recomendada (RDI) debido a la dificultad de evaluar/definir la dosis de polifenol que provoca una determinada actividad positiva. Así la EFSA, a raíz de una petición de la Comisión Europea, ha

emitido una opinión científica sobre una solicitud de alegación de salud para los polifenoles del aceite de oliva en virtud de la artículo 13 del Reglamento (CE) nº 1924/2006. EFSA considera que la evidencia proporcionada era insuficiente para establecer una relación de causa y efecto entre el consumo de polifenoles de aceite de oliva y el mantenimiento de las concentraciones de colesterol de HDL normal en sangre. Esto no afecta a la tendencia actual a incrementar el consumo de alimentos ricos en polifenoles y la aparición de trabajos científicos que avalan sus efectos beneficiosos.

Otros efectos biológicos atribuidos a los polifenoles incluirían su actividad **antimicrobiana**, su actividad **anti-inflamatoria**, su efecto **prebiótico**, su efecto **vasodilatador** y su consideración como **anti-nutrientes** (Landete, 2012).

1.5.4. Compuestos fenólicos en sub-productos de la industria alimentaria

Los residuos agrícolas e industriales son una fuente interesante de antioxidantes naturales. Se han identificado un gran número de compuestos fenólicos con actividad antioxidante en sub-productos agrícolas: en las cáscaras de cebada, lino (Hao y Beta, 2012), castaña y avellana (Nazzaro y col., 2012); en las pieles de la patata (Mohdaly, 2013) y de pistachos (Tomaino y col., 2010).

Los sub-productos del brócoli (restos de la cosecha como hojas y tallos) también han mostrado ser una fuente de compuestos fenólicos. Las hojas del cultivar Viola presentaron un contenido en fenoles de 163.18 mg/g (peso seco) (Domínguez-Perles y col., 2010). Las pieles y semillas del tomate han mostrado tener mayor contenido en fenoles que la pulpa (George y col., 2004; Toor y Savage, 2005). Recientemente Ćetković y col., (2012) analizaron el residuo del procesado del zumo de tomate encontrando contenidos de fenoles en sus extractos de hasta 5206 µg/g. Además de su actividad antioxidante estos sub-productos del tomate mostraron una alta actividad anti-proliferativa.

El procesado de frutas da como resultado una gran cantidad de sub-productos dependiendo de la materia prima. En la **Tabla 8** se muestran algunos datos

interesantes sobre el rendimiento de estas industrias. Gorinstein y col., (2001) encontraron que el contenido total de fenoles en las pieles de limones, naranjas y pomelo era un 15% superior que en sus correspondientes frutas peladas. Igualmente dichos autores encontraron que la cantidad de fenoles en otras frutas como manzanas, melocotones y peras era el doble que en la cantidad hallada en las peladas. Sub-productos de varias frutas tropicales (pitanga, acerola, marañón y piña) también han mostrado poseer un gran contenido en compuestos fenólicos (2787.09-12696.03 mg de equivalentes de ácido gálico (GAE)/100 g en peso seco) (da Silva y col., 2014). Recientemente Mena y col., (2014) han encontrado en el poso del vino de granada (sub-producto de la elaboración de vino) un contenido total de fenoles de 3000 mg/100 g (peso seco), predominantemente en forma de taninos hidrolizables, antocianos, ácido elágico y ácido gálico.

Tabla 8. Cantidad anual mundial procesada y sub-productos resultantes de ciertas frutas.

Fruta	Procesado anual (millones de toneladas)	Sub-productos (%, base húmeda)	Residuos anuales estimados
Cítricos	31.2	50	15.6
Manzana	12.0	25-35	3.0-4.2
Pera	1.7	n.d.	---
Melocotón[a]	1.0	n.d.	---
Uva	50.0	15-20	5.0-9.0
Plátano[b]	30.0	30	9.0
Kiwi	1.0	30	< 0.3

[a] En conserva, [b] cocido.
Abreviaturas: n.d., no disponible.
Fuente: Djilas y col., (2009).

1.5.5. Extractos vegetales ricos en compuestos fenólicos como antioxidantes alimentarios

El uso de antioxidantes en productos alimentarios está controlado por las leyes de cada país o estándares internaciones. Aunque se han propuesto muchos compuestos como potenciales antioxidantes en la inhibición de la deterioración oxidativa sólo unos pocos pueden usarse en alimentos. En la Unión Europea la regulación de los antioxidantes está estipulada por el Reglamento (CE) nº 1333/2008 del Parlamento Europeo y del Consejo, de 16 de diciembre de 2008, sobre aditivos alimentarios

(última modificación 13 de febrero de 2014). Antioxidantes sintéticos han sido ampliamente utilizados en alimentos pero cada vez la industria alimentaria, en respuesta a la demanda de productos naturales por parte de los consumidores, está activamente en busca de soluciones para minimizar el enranciamiento oxidativo y aumentar la vida útil de los productos alimentarios (Karre y col., 2013). Además recientes publicaciones revelan que el uso de polifenoles naturales en alimentos y bebidas ha aumentado en un 69% desde 2009, siendo las categorías más populares bizcochos y galletas (10.7% de los lanzamientos entre 2009 y 2013) seguidas de pastillas, gelatinas y masticables (7.87%) y tartas, pasteles y productos de pastelería (6.66%). Los tés y zumos de frutas también son muy populares (Starling, 2014).

La actividad antioxidante de los compuestos fenólicos extraídos de diferentes fuentes ha sido estudiada en diferentes alimentos y sistemas alimentarios modelo. Wijeratne y col., (2006) evaluaron la actividad antioxidante de varios extractos de sub-productos de almendra en varios sistemas alimentarios. El extracto de la cáscara verde demostró una mayor capacidad antioxidante inhibiendo la formación tanto de productos primarios y secundarios de la oxidación de un sistema modelo de aceite de maíz. . Ajila y col., (2010) observaron 22 veces más actividad secuestrante de radicales con la incorporación de 7.5% de extracto de piel de mango en la preparación de macarrones. Mildner-Szkudlarz y col., (2013) propusieron la utilización de pulpa de uva blanca como aditivo en la elaboración de galletas (hasta un 30% de adición a la harina) mostrando una mayor actividad antioxidante. Su capacidad secuestrante de radicales aumentó cerca de seis veces en comparación a las galletas control. La incorporación de polvo de cáscara de granada (10 mL) en la elaboración de pasteles de carne de cabra redujo la formación de TBARS hasta en un 67% (Devatkal y col., 2010). El extracto de la semillas de uva tiene un potencial antioxidante 20 y 50 veces mayor al de la vitamina E y C respectivamente (Carpenter y col., 2007). Numerosos estudios han concluido que dicho extracto es un efectivo antioxidante natural tanto en carne cruda como cocida. En el estudio llevado a cabo por Ahn y col., (2007) el uso de un 1% de extracto de semilla de uva inhibió en un 92% la formación de sustancias reactivas del ácido tiobarbitúrico (TBARS) en carne picada. Lee y col., (2006) examinaron el potencial de diferentes clases de polifenoles presentes en la frambuesa en la inhibición de la

oxidación lipídica en carne de pavo mecánicamente separada y en carne de cerdo picada y cocida. La carne de pavo mecánicamente separada tratada con un 0.32% de polvo de zumo de frambuesa mostró inhibición de la oxidación lipídica. Las catequinas del té demostraron ser más eficientes que el α-tocoferol en la inhibición de la oxidación del músculo de carne picada de carne de ave y pescado frescos (ambos aplicados a 300 mg/kg) (Tang y col., 2001). La adición de un 2% de fibra de naranja a salchichas retardó significativamente el desarrollo de TBARs durante 28 días de almacenamiento en refrigeración en comparación a las salchichas no tratadas (Fernandez-López y col., 2004). En el estudio llevado a cabo por Viuda-Martos y col., (2009) la introducción de las aguas de lavado de cítricos, en concreto de naranjas, en la formulación de una mortadela aumentó su vida útil. La adición de un 5-10% del agua de lavado junto con aceites esenciales de orégano y tomillo reduce el grado de oxidación lipídica de la mortadela.

1.6. NECESIDAD DE EXPLORAR NUEVOS INGREDIENTES

El aprovechamiento de subproductos agrícolas es un tema de gran interés a nivel internacional, hay una tendencia a obtener producciones sin residuos llevando a cabo el reciclado y aprovechamiento de materiales recuperables, desperdicios y subproductos (Larrauri, 1995). El aprovechamiento de estos materiales además de evitar trastornos medioambientales, aporta una mayor rentabilidad económica al proceso industrial de partida. Además, una rentabilización de la gestión de los residuos generaría nuevas industrias de todo tipo, con las consiguientes ventajas sociales que ello reportaría (Martín, 1995; Cháfer y col., 2000). El objetivo de la industria agroalimentaria en este campo es transformar sus subproductos y residuos en co-productos.

Entre estos subproductos se encuentran las **aguas de lavado**. Estas aguas pueden provenir de las etapas de lavado, escaldado de las frutas o bien de la obtención de otro subproducto agrícola, como es la fibra. Para la realización de estos procesos es necesario generar un gran volumen de agua, un bien que escasea, y una buena forma de minimizar los problemas económicos y medioambientales que ello ocasiona es

reutilizarla en la industria alimentaria. Por otro lado, esta agua contiene compuestos potencialmente beneficiosos tanto desde un punto de vista tecnológico como de salud (Viuda-Martos y col., 2009). La industria transformadora de zumos de frutas genera una gran cantidad de estas aguas de lavado, que se podrían revalorizar mediante la extracción de sus compuestos bioactivos. Viuda-Martos y col., (2008) caracterizaron las aguas de lavado de cítricos y determinaron que estas aguas de lavado adicionadas a productos cárnicos cocidos provocan un descenso en los niveles de nitrito residual de aproximadamente un 40%.

Los extractos vegetales, debido a su compleja composición, poseen potencialmente mayor poder antioxidante que compuestos aislados individualmente. Su potencial para combatir diferentes enfermedades (cáncer, infecciones, envejecimiento prematuro, etc.) reside en el papel que ejercen en la lucha contra radicales libres que, entre otros, son causa de numerosos cambios negativos en la piel. Es por este motivo que resulta de gran interés explorar la aplicación de nuevos ingredientes en la formulación de alimentos que puedan aportar potenciales efectos beneficiosos para la salud, a la vez que se mejora la rentabilidad de los actuales procesos industriales.

Por otro lado la incorporación de frutas en productos lácteos de consumo habitual no sólo ayuda a darle un valor añadido y a aumentar la diversificación del producto final sino que también aporta beneficio a la reducción en pérdidas post-cosecha y por tanto a las pérdidas económicas de las industrias agroalimentarias. Para los consumidores la asociación de fruta y producto lácteo fermentado es percibido como muy saludable, por lo que las dos categorías conformarían una categoría híbrida de productos lácteos ofreciendo salud, sabor y provecho (Khurana y Kanawjia, 2007).

En último lugar se debe tener en mente que en presencia de todos estos nuevos componentes la estructura del gel así como otras propiedades de las leches fermentadas cambiarán y será necesario un estudio en profundidad.

1.6.1. Dátil

La palmera datilera (*Phoenix dactylifera* L.) es uno de los cultivos comerciales más importantes. Se cultiva predominantemente en la región árabe y en la zona fronteriza con la costa mediterránea (Aslam y col., 2013). La producción mundial de dátil en 2012

fue de 7.548.918 t de las cuales 16.935 t se produjeron en Europa, lo que supuso un incremento del 10.49% con respecto a 2011. En España se estimó una producción de 4.000 t para el año 2012 (Food and Drug Organization (FAO), 2014). Sin embargo se sabe que una gran cantidad de dátiles son desechados (2.000.000 anuales en el mundo) debido a que criterios de calidad (tamaño insuficiente, defectos de apariencia, daños en el fruto) (Besbes y col., 2009) y durante su clasificación, almacenamiento y acondicionado (Ahmed y col., 2013). Esto supone una gran pérdida económica ya que estos dátiles siguen siendo ricos en compuestos bioactivos.

La importancia económica de la palmera datilera radica en su valorado fruto, el cual se compone de 44-88% de azúcar, grasa (0.2-0.5%), minerales como el potasio, calcio, magnesio y hierro, proteínas (2.3-5.6%), fibra dietética (6.4-11.5%) así como vitaminas y amino ácidos (Al-Shahib y Marshall, 2003). Además estudios preclínicos muestran que el dátil posee actividad secuestrante de radicales libres, antioxidante, anti-mutagénica, antimicrobiana, anti-inflamatoria, gastroprotectora, hepatoprotectora, nefroprotectora, anti-cancer e immunoestimulante (Baliga y col., 2011).

En nuestro entorno se encuentra el palmeral de Elche, que cuenta con más de 200.000 palmeras y una producción media de 4.500 t (de forma que la producción nacional y local nacional es equiparable). Pero por desgracia se estima que de la media de 4.500 t tan sólo se aprovechan cerca de 100 t. El dátil mayoritariamente producido en el palmeral es el tipo Negre o Dorat, que se corresponde con un dátil de tipo blando (según la clasificación internacional). Se caracteriza por tener un alto contenido en agua (55-60%) y elevado contenido en azúcar (70% sobre base seca), lo que lo hace altamente perecedero, por lo que no es posible el aprovechamiento comercial rentable de todo el dátil fresco producido en Elche. Por todo ello es necesaria una investigación y puesta a punto tecnológica, ya no sólo para aprovechar el producto en fresco, sino para dar salida al exceso de producción mediante su incorporación como posible ingrediente funcional por su elevado valor nutritivo.

Se sabe que los dátiles pasan por una serie de etapas de maduración. Estos estados comprenden: 'kimri' (inmaduro; contenido en humead del 85%), 'khalal' (crujiente, 50-60% humead), 'rutab' (maduro, blando, 35-40% humedad) y 'tamr' (maduro, reducida

humedad sobre un 20%) (Biglari y col., 2008) y equivalen aproximadamente al verde, rojo y negro. El-Zoghbi (1994) demostró que el contenido en fibra dietética de los dátiles disminuye desde un 13.7% en el primer estadío de maduración a 3.6% en el cuarto estadío (dátiles negros secos). Esto sugiere que hay un mayor beneficio al consumir los dátiles frescos que secos (Al-Shahib y Marshall, 2002).

Diversos estudios se han llevado a cabo centrados en las propiedades funcionales del dátil y sus sub-productos. Al-Farsi y col. (2007) estudiaron las características funcionales de tres variedades de dátiles secos cultivados en Omán así como sus co-productos. De todos los productos analizados (carne de dátil, jarabe de dátil, torta de prensado y semillas) las semillas son el subproducto que posee el mayor contenido en fenoles totales (3102-4430 mg de GAE/100 g en peso fresco), seguido de la torta de prensado (165-435 GAE/100 g). Del mismo modo la actividad antioxidante es mayor en las semillas (580-929 µmoles de equivalentes de Trolox (TE)/g en peso fresco).

En un estudio publicado recientemente se ha evaluado la calidad de dátiles de textura dura (dátiles de segunda calidad) de tres variedades (Deglet Nour, Allig y Kentichi). Todos ellos mostraron el mismo contenido en azúcar (73.30-89.55 g/100g en base seca), fibra (7.95-18.83 g/100g en base seca) y fenoles totales que los de elevada calidad. Además al adicionar estos ingredientes a mermelada, y más concretamente la variedad Allig, los resultados mostraron una reducción de azúcares y un aumento de la firmeza así como de la capacidad de retención de agua (Besbes y col., 2009).

Por lo que los co- y sub-productos de la industria del dátil pueden ejercer como fuente de fibra dietética, polifenoles totales y actividad antioxidante, y podrían ser potencialmente considerados como fuente de antioxidantes naturales.

1.6.2. Membrillo

El membrillero es un frutal de la familia de las rosáceas, en concreto perteneciente al género *Cydonia*, el cual tiene una única especie denominada *Cydonia oblonga*, el membrillero, de la que se conocen más de treinta variedades distribuidas por Europa, Asia y América (Junta de Andalucía, 2013). Las zonas más consumidoras y productoras de membrillos a nivel mundial son Turquía y China. La producción mundial de membrillo en 2012 fue de 596.532 t, de las cuales 14.000 t se produjeron en España

(FAO, 2014). Extremadura, Andalucía, Castilla La Mancha, Cataluña y Murcia son las comunidades autónomas más productoras.

El consumo en fresco del fruto no es común debido a su sabor áspero y la dureza de su pulpa. Por lo que tradicionalmente estaba destinado a la elaboración de conservas, mermeladas, jaleas, dulces, compotas, gelatinas, sorbetes o licores de mesa. Es una fruta rica en sales minerales y vitaminas y las pepitas del fruto, muy numerosas, contienen abundante mucílago, el cual posee propiedades laxantes. El membrillo tiene un bajo contenido en grasa y es una fuente importante de ácidos orgánicos, azúcares, fibra y minerales como el potasio, fósforo y calcio (Silva y col., 2004; Rodríguez-Guisado y col., 2009).

El membrillo está constituido por 90.6% de pulpa, 4.4% de piel y 5.0% de núcleo con semillas (Sharma y col., 2011). La pulpa y piel de membrillo son fuente de compuestos fenólicos y presentan propiedades antioxidantes y potencialmente antimicrobianas. Los extractos de la piel de membrillo muestran un contenido en fenoles totales entre 105-157 mg/100g y entre 37-47 mg/100g en el caso de la pulpa (Fattouch y col., 2007). Un análisis comparativo entre el potencial antimicrobiano del extracto de membrillo en un rango de cepas de microorganismos (*Staphylococcus aureus*, *Pseudomonas aeruginosa* y *Bacillus cereus*) mostró una importante actividad antimicrobiana. Las concentraciones mínimas inhibitorias y bactericidas se obtuvieron para concentraciones de 102 a 104 µg de polifenol/mL (Fattouch y col., 2008). Las semillas también poseen una gran cantidad de compuestos bioactivos tales como ácidos orgánicos (ácidos cítrico, ascórbico, málico, quínico, shiquímico y fumárico), aminoácidos (ácido glutámico, ácido aspártico y asparagina, como los más abundantes) y un contenido en compuestos fenólicos de hasta 116.4 mg/kg, siendo el ácido 5-*O*-cafeoilquinico el más abundante (Silva y col., 2005).

El membrillo también se ha usado para enriquecer otros productos. En el estudio llevado a cabo por Wojdylo y col., (2008) resultó que la adición de un 10% de extracto de membrillo a mermelada de fresa durante su preparación aumentaba el contenido en componentes fenólicos en el producto final, especialmente proantocianidinas.

En cuanto a sus propiedades terapéuticas estudios han demostrado que la administración de extracto de membrillo produce un efecto anti-ulcerativo en ratas. Dicho efecto se atribuye a sus compuestos fenólicos (dosis entre 5-10 mg de fenoles semi-purificados suprimen la inducción de úlcera en ratas) que pueden tener beneficios sobre la salud actuando en vasos sanguíneos y en el tracto gastrointestinal (Hamauzu y col., 2006; Hamauzu y col., 2008). Un reciente estudio propone el uso de un extracto acuoso de membrillo ('quince hot water') como ingrediente funcional por su efecto antialérgico sobre los síntomas de alergia tipo I probados en ratas y en células *in vitro* (Shinomiya y col., 2009). Recientemente Khoubnasabjafari y Jouyban (2011) han realizado una profunda revisión sobre la bioactividad del membrillo (diferentes partes, extractos y fitoquímicos) *in vitro* destacando su actividad anti-radicales, actividad anti-proliferativa, actividad anti-hemolítica y actividad anti-alérgica y en modelos animales probando su efecto hipolipidémico, efecto protector renal, efecto antidiabético y efecto antialérgico.

1.6.3 Granada

La familia de la granada tiene un único género *Punica* con dos especies *P. granatum* y *P. protopunica*. Esta última está considerada como el antepasado del género *Punica* el cual puede haber contribuido al proceso evolutivo de la forma cultivada de la granada (Teixera da Silva y col., 2013). La granada es nativa de los Himalayas en el noreste de India, pero se ha cultivado y naturalizado a través de Oriente Medio, de toda la región Mediterránea, de las partes más secas del sureste de Asia, noreste de África y África tropical y hasta cierto punto de Estados Unidos, concretamente a California y Arizona (Viuda-Martos y col., 2010). A nivel mundial India e Irán son los principales productores con 900.000 y 800.000 toneladas (Medjakovic y Jungbauer, 2013). En términos de producción España es la líder (18.5 t/ha) seguida por Estados Unidos (18.3 t/ha). A pesar de que España cuenta con un área de cultivo muy pequeña (2000 ha) su cuota de exportación es del 37.8% de la producción total (37.000 t) seguida por Israel (23.5%) y Estados Unidos (15.5%) mientras que India tiene la menor cuota (Teixera da Silva y col., 2013).

La parte comestible de la granada (50%) se compone de los arilos (40%) que se utilizan para elaborar el zumo y de semillas (10%). El otro 50% se corresponde con la piel, la cual es fuente importante de compuestos fenólicos como flavonoides, elagitaninos y proantocianidinas (Viuda-Martos y col., 2010). Las semillas son ricas en lípidos; el aceite de la semilla comprende del 12-20% del peso total de la semilla y consiste en un 80% en ácidos grasos conjugados octadecatrienoicos, con un elevado contenido en ácido punícico (*cis*-9, *trans*-11, *cis*-13). Otros componentes minoritarios incluyen esteroles, esteroides y cerebrosidos (Faria y Calhau, 2011). Los arilos se componen de un 85% agua, 10% de azúcares totales (mayoritariamente fructosa y glucosa), 1.5% pectinas y ácidos orgánicos como ascórbico, cítrico y málico, además de compuestos fenólicos (Aviram y col., 2000; Tezcan y col., 2009). Las antocianinas son el mayor y más importante grupo de flavonoides presentes en los arilos, responsables de su color rojo. Además de los antocianos en los arilos se encuentran otros compuestos fenólicos como ácidos gálico, elágico, cafeico, clorogénico y *p*-coumárico (Viuda-Martos y col., 2010). Además el zumo de granada presenta un mayor contenido en fenoles totales, y presumiblemente una mayor actividad antioxidante, que otros zumos de frutas como el de uva, mango y naranja, entre otros (Mahdavi y col., 2010) e incluso sus sub-productos han mostrado poseer un gran potencial antioxidante (Viuda-Martos y col., 2011).

Los efectos saludables del fruto de la granada así como el zumo y sus extractos han sido ampliamente estudiados en estudios modelo en ratas y humanos en relación a una gran cuantía de enfermedades crónicas. Entre las bioactividades atribuidas a la granada se encuentran: protección vascular (actividad antioxidante, anti-hipertensiva, regulación lipídica), protección digestiva (gastroprotección, hepatoprotección, anti-diarrea, anti-helmintos), actividad antipatogénica (anti-bacteria, antivirus), actividad anticancer (anti-inflamación, anti-angiogénesis, inducción de la apoptosis, inhibición de la proliferación y de la invasión), actividad antidiabetes, inmunomodulación y antiobesidad, entre otros (Aviram y Dornfeld, 2001; Fuhrman y Aviram, 2007; Basu y Penugonda, 2009; Stowe, 2011; Viuda-Martos y col., 2010; Wang y col., 2010; Betanzos-Cabrera y col., 2011; Viladomiu y col., 2013).

1.7. ÁCIDO LINOLEICO CONJUGADO COMO INGREDIENTE FUNCIONAL

1.7.1. Enfermedades cardiovasculares y obesidad

Las enfermedades cardiovasculares (ECV) son la primera causa de muerte en todo el mundo. En 2008 se registraron a nivel mundial 17.3 millones de muertes por esta causa, lo que representa el 30% del total de muertes registradas. Se calcula que en 2030 morirán cerca de 23.3 millones de personas por ECV, y se prevé que sigan siendo la principal causa de muerte (World Health Organization (WHO), 2013a). En Europa las ECV fueron la principal causa de muerte (36% de las muertes registradas), mientras que en España fue la segunda (28% de las muertes registradas) sólo por detrás de las muertes registradas por neoplasias (tumores y cáncer) (Eurostat, 2010).

El sobrepeso y obesidad[1] son el quinto factor principal de riesgo de defunción en el mundo y es un problema creciente tanto en países desarrollados como en vías de desarrollo. Un billón de adultos en el mundo padecen sobrepeso y más de 300 millones son obesos. Al menos 2.8 millones de personas mueren cada año a causa del sobrepeso u obesidad (WHO, 2013b). En España el 62.8% de los hombres y el 44.6% presentaban sobrepeso u obesidad en el año 2009, situándose entre los países europeos con una mayor tasa (OECD, 2011). Según la Organización para la Cooperación y el Desarrollo Económicos (OCDE) dos de cada tres hombres presenta sobrepeso y una de cada seis personas padece obesidad. La OCDE prevé además que la proporción de adultos con sobrepeso puede aumentar aproximadamente un 10% más en los próximos años (OECD, 2012). La obesidad está involucrada en numerosos problemas metabólicos, ya que afecta a procesos como el control de glucosa, lípidos y presión sanguínea. Es, por lo tanto, el origen de un conjunto de condiciones conocido como síndrome metabólico (Misra y Khurana, 2008).

Para reducir la carga de las ECV y la obesidad, la OMS recomienda realizar actividad física regular, evitar el consumo de tabaco y alcohol, limitar la ingesta energética y consumir una dieta equilibrada (WHO, 2013a). Resulta evidente que la composición de la dieta influye de manera directa sobre muchos factores de riesgo asociados a las ECV

[1] En base a los criterios de la OMS se entiende como personas con sobrepeso a aquellas con un índice de masa corporal (IMC) igual o superior a 25 y personas con obesidad a aquellas con un IMC igual o superior a 30.

y la obesidad. Debido a que la composición de la grasa influye de manera determinante en la progresión de patologías como la aterosclerosis, en general, se recomienda disminuir el consumo de ácidos grasos saturados (AGS), ácidos grasos *trans* (AGT) y colesterol, y sustituirlos por ácidos grasos monoinsaturados (AGMI) y AGPI (Aranceta y Pérez-Rodrigo, 2012).

Este mencionado incremento en la incidencia de la obesidad está creando una presión por parte de los consumidores y supone una oportunidad única para la industria alimentaria de desarrollar alimentos funcionales que ayuden a la prevención y/o tratamiento de estas patologías (Picó y col., 2006). Los lípidos están entre los compuestos bioactivos que han recibido más atención (tanto en términos cuantitativos como cualitativos) en el desarrollo de alimentos más saludables, especialmente entre los productos cárnicos (Jiménez-Colmenero, 2007). Además se ha observado que la calidad de los lípidos de la dieta podría ser un importante modulador en términos de morbilidad y mortalidad en las enfermedades relacionadas con el estilo de vida (Nagao y Yanagita, 2005).

1.7.2. Implicación del ácido linoleico conjugado en la salud

Mientras que los ácidos grasos 'no conjugados' *trans* (principalmente provenientes de aceites vegetales parcialmente hidrogenados) pueden influir de forma negativa en la salud humana, los ácidos grasos conjugados, principalmente CLA, han demostrado tener gran variedad de efectos biológicos beneficiosos (Dilzer y Park, 2012). De hecho hoy en día el CLA está excluido de la definición dada por el Codex Alimentarius para los ácidos grasos *trans*, en base a la evidencia (principalmente por estudios preclínicos) de sus efectos beneficiosos sobre la prevención del cáncer y el control del peso (Wang y Proctor, 2013).

El CLA (**Figura 3**) es el término colectivo utilizado para un grupo de isómeros posicionales y geométricos del ácido linoleico con un sistema de dobles enlaces conjugados (Moon y col., 2008). La familia del CLA puede incluir hasta 28 isómeros posibles diferentes; conociéndose en dos de ellos (*cis*-9, *trans*-11-CLA y *trans*-10, *cis*-12-CLA) bioactividad. El isómero *cis*-9, *trans*-11-CLA es el más predominante, presente de forma natural como ácido graso esterificado en la molécula de triglicérido de la

grasa de rumiantes y por las bacterias de los rumiantes y conversión *in vivo* a partir del ácido *trans*-11-vacénico en el hígado y tejido adiposo de los rumiantes (Palmquist y col., 2005). Además de estar presente en los productos derivados de los rumiantes (leche, productos lácteos, carne y productos cárnicos) podemos disponer de CLA comercial en forma de suplementos enriquecidos (normalmente con una formulación del 80% a una ratio 1:1 de los isómeros *cis*-9, *trans*-11-CLA y *trans*-10, *cis*-12-CLA) producidos a partir de aceite de girasol rico en ácido linoleico (McCrorie y col., 2011). En España la ingesta media de CLA de fuentes naturales (no suplementadas) procedente de rumiantes es de 140 mg/día siendo superior en otros países como Suecia o Francia (**Tabla 9**).

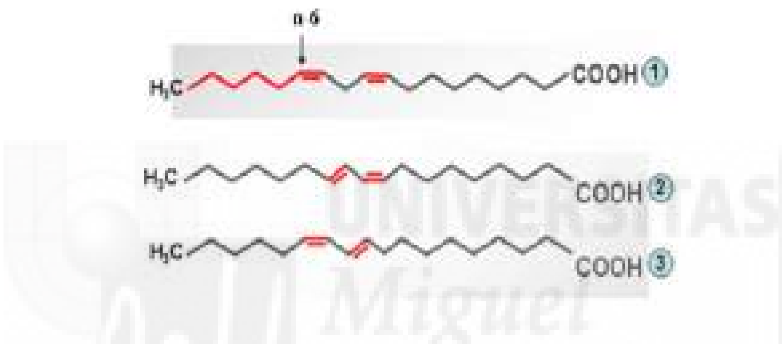

Figura 3. Estructura del ácido linoleico y sus principales CLA derivativos: 1: Ácido linoleico; 2: *cis*-9, *trans*-11-CLA; 3: *trans*-10, *cis*-12-CLA. Fuente: Benjamin y Spener, (2009).

Tabla 9. Ingesta dietética de ácidos grasos *trans* (AGT) procedente de rumiantes[1].

País	AGT (g/día)	*cis*-9, *trans*-11-CLA (g/día)
Dinamarca	1.7	0.25
Francia	1.7	0.30
Alemania	1.7	0.28
Países Bajos	1.2	0.23
Nueva Zelanda	1.1	n.d.
Australia	1.0	n.d.
España	1.3	0.14
Suecia	1.3	0.33
Italia	1.2	0.22
Estados Unidos	1.2	0.18
Reino Unido	0.98	0.21
Grecia	0.8	0.15

Fuente: Gebauer y col., (2011).

Existen diferencias según la fuente de CLA (rumiante o suplementos) y estas incluyen: distribución de los isómeros, nivel de consumo, distribución regio-específica en la molécula del triglicérido y biodisponibilidad (Wang y Proctor, 2013). Como se ha mencionado el CLA comercial contiene dos isómeros en abundancia (*cis*-9, *trans*-11-CLA y *trans*-10, *cis*-12-CLA) mientras que en los alimentos derivados de los rumiantes (tales como carne de vacuno, oveja y productos lácteos) el isómero predominante es el *cis*-9, *trans*-11-CLA (70-90%) encontrándose el *trans*-10, *cis*-12-CLA a nivel de trazas (Lock y Bauman, 2004). En la Tabla 10 se presentan los contenidos de CLA y de AGT en productos de consumo habitual.

Tabla 10. Cantidad de CLA en productos procedentes de rumiantes de consumo habitual.

Alimento	Grasa total (g/100 g)	Total CLA (mg/g grasa)[1]
Productos lácteos		
Queso cheddar	36.4	3.6 (93)
Leche entera	3.10	5.5 (92)
Yogur	1.16	4.4 (86)
Carnes		
Ternera picada, 20.8% grasa	21	4.3 (85)
Ternera picada, 22.1% grasa	22.1	4.3 (85)

[1] Los valores en paréntesis representan el porcentaje de cis-9, 11-trans isómero en CLA total.
Fuente: Gebauer y col., (2011).

Multitud de investigaciones han mostrado la asociación del CLA con potentes propiedades anti-carcinogénicas, anti-inflamatorias, anti-ateroscleróticas, anti-diabetes y anti-obesidad tanto *in vitro* como *in vivo* y cómo su eficacia frente a una condición particular puede variar considerablemente entre los isómeros individuales (Wahle y col., 2004; Igarashi y Miyazawa, 2005; Bhattacharya y col., 2006; Tsuzuki y col., 2007; Kee y col., 2010). Los mecanismos por los cuales proporcionan estas propiedades beneficiosas van desde su habilidad para modular la expresión de los genes asociados con enfermedades patogénicas hasta su habilidad para competir con ácidos grasos omega-6 pro-inflamatorios, como ácidos linoleico y araquidónico, en su incorporación en la membrana celular (Destaillats y col., 2005; Plourde y col., 2006). Aunque ciertas investigaciones revelan cambios metabólicos indeseables (Andreoli y col., 2009). A continuación se muestra una tabla resumen con las principales bioactividades del CLA, mecanismo propuesto así como su nivel de eficacia (**Tabla 11**).

Tabla 11. Ejemplos de los estudios realizados sobre los efectos saludables del ácido linoleico conjugado (CLA) en modelos animales y humanos.

Actividad biológica	Ejemplos de estudios en modelos animales	Mecanismo propuesto	Evidencias en animales	Ejemplos de estudios en humanos	Evidencias en humanos
Anticancerígeno	Kelley y col., (2007); Bhattachayra y col., (2006); Lee y col., (2005).	El CLA puede estar implicado en la reducción de la producción de eicosanoides, interfiriendo con las vías de señalización celular, inhibiendo la síntesis de ADN, aumentando la apoptosis así como inhibiendo la angiogénesis.	+++	Larsson y col., (2005); McCann y col., (2004); Chajes y col., (2003); Rissanen y col., (2003); Voorrips y col., (2002); Aro y col., (2000).	+
Prevención de ECV	Bhattachayra y col., (2006); Kritchevsky y col., (2004); McLeod y col., (2004); Kritchevsky, (2000); Lee y col., (1998); Nicolosi y col., (1997).	CLA reduce el colesterol total, TG, el colesterol LDL y aumenta el colesterol HDL. CLA participa con el receptor PPAR (clave para la lipogéneis), con las proteínas SREBPs (clave en la síntesis y elongación de los AG) y SCD (clave en la formación de TG y colesterol).	+++	Attar-Bashi y col., (2007); Iwata y col., (2007); Herrera y col., (2005); Berven y col., (2000).	Cambios en colesterol, LDL, TG y NEFA: - Descenso hipertensión: +++
Reducción de la grasa corporal	Park y Pariza, (2007); Meadus y col., (2002); Azain y col., (2000); DeLany y West, (2000); Park y col., (1999).	Resultado de multiples mecanismos: aumento del gasto energético, reducción de la acumulación lipídica en tejido adiposo, aumento de la apoptosis de adipocitos, modulación de adipokinas y citoquinas tales como leptina y TNF-α e incrementando la β-oxidación de los AG en músculo esquelético.	+++	Onakpoya y col., (2012), Schoeller y col., (2009); Park y Pariza, (2007); Whigham y col., (2007); Bhattachayra y col., (2006); Gaullier y col., (2004), (2005); Brown y McIntosh, (2003); Smedman y Vessy, (2001); Thom y col., (2001); Blankson y col., (2000).	++
Respuesta inmune e inflamatoria	Bhattachayra y col., (2006); Poirier y col., (2006); Changhua y col.,	Respuesta anti-inflamatoria: reducción de la inflamación colónica, modulación de la producción de citoquinas,	++	Turpeinen y col., (2008); Song y col., (2005); Tricon y col., (2004); Albers y col., (2003).	++

	(2005); Bassaganya-Riera y col., (2002), (2003); Luongo y col., (2003); Yang y Cook, (2003); Yu y col., (2002).	prostaglandinas y leucotrieno B4. Respuesta inmune: modulación de TNF-α, citoquinas, prostaglandinas, NO, y reducción de las respuestas inmunes de tipo alérgico.			
Salud ósea	Banu y col., (2006); Berge y col., (2004); Weiler y col., (2004); Kelly y col., (2003).	Mejora la masa ósea, aumenta la absorción de calcio, disminuye la osteoclastogénesis.	**+**	Park y col., (2008); Brownbill y col., (2005); Doyle y col., (2005); Jewell y col., (2005); Jewell y Cashman, (2003).	**++**

Abreviaciones: ECV, enfermedades cariovasculares; TG, triacilglicéridos; LDL, lipoproteínas de baja densidad; HDL, lipoproteínas de alta densidad; PPAR, *peroxisome proliferator-activated receptor*; SREBPs, *sterol regulatory element-bindings*; SCD, estearoil-CoA desaturasa (*steroyl-CoA desaturase)*; TNF-α , factor de necrosis tumoral alfa; NEFA, ácidos grasos no esterificados (*non-esterified fatty acids*); NO, óxido nitroso.

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1.7.3. Ácido linoleico conjugado en leches fermentadas

La grasa de la leche es la mayor fuente animal de CLA y su contenido varía desde 2 hasta 53.7 mg/g grasa (Collomb y col., 2006). Esta amplia variación de su contenido se debe a factores tales como la alimentación, ubicación del animal y la raza de los animales (Kee y col., 2010). Los productos lácteos son por tanto la fuente más importante de CLA en productos de origen animal y su contenido varía de 3.59 a 7.96 mg/g de grasa (Lin y col., 1995). La variabilidad en el contenido en CLA en quesos, yogures y otros productos lácteos comerciales depende del contenido inicial de CLA de la leche, de los cultivos iniciadores, tiempo transcurrido desde su elaboración, tratamiento térmico, calidad de las proteínas (Xu y col., 2004). En los productos lácteos el CLA es producido por ciertos microorganismos del rumen tales como las especies de *Butyrivibrio* (Ogawa y col., 2005). Pero además de las bacterias del rumen algunos cultivos estárter como especies de *Propionibacterium* y *Lactobacillus* son capaces de producirlo a partir de ácido linoleico (Jiang y col., 1998).

Incrementar el contenido en CLA en componentes de la dieta habituales es una potencial via para aumentar la ingesta de CLA y los productos lácteos son unos idóneos candidatos como vehículos del CLA debido a la habilidad de las bacterias acido lácticas para producir CLA a partir de ácido linoleico (Jiang y col., 1998). Numerosos estudios se han centrado en la capacidad para producir CLA por parte de bacterias probióticas en sistemas modelo. Estos sistemas modelo utilizan una fuente de ácido linoleico, normalmente aceites vegetales como el de girasol o cártamo, para producir CLA o directamente se les adiciona ácido linoleico libre. En el estudio llevado a cabo por Lin y col., (1999) seis cultivos lácticos de lactobacilos, lactococcos y estreptococos fueron capaces de producir CLA *in vitro* en leche desnatada esterilizada a partir de ácido linoleico libre. Dos cepas de *L. acidophilus* y *L. casei* produjeron CLA a partir de ácido linoleico en MRS caldo y en leche desnatada suplementados con ácido linoleico (Alonso y col., 2003). Xu y col., (2004) demostraron que la capacidad de las LAB para producir CLA a partir de ácido linoleico depende tanto de la cepa utilizada como de la fuente lipídica; las 11 cepas que analizaron fueron capaces de producir CLA pero solo a partir de aceite hidrolizado.

Además el hecho de que numerosos productos fermentados contengan niveles superiores de CLA que sus homólogos no fermentados ofrece la posibilidad de elaborar productos lácteos fermentados con altos niveles de CLA mediante el uso de bacterias probióticas y LAB. Con lo que la identificación de LAB capaces de producir CLA a partir de una fuente de ácido linoleico sería de gran interés en la producción de productos lácteos funcionales para el consumo humano (Rodríguez-Alcalá y col., 2011).

Por lo tanto, el contenido en CLA de la leche puede incrementarse mediante la modificación de la dieta del animal y las condiciones de producción de la leche o una alternativa a estos medios sería mediante la adición de un precursor a la leche (ácido linoleico) y una cepa bacteriana productora de CLA (Meraz-Torres y Hernández-Sánchez, 2012).

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Resulta de interés tanto la incorporación de extractos vegetales y zumos ricos en polifenoles como la potenciación del contenido en CLA para desarrollar leches fermentadas con potenciales efectos saludables. El primer paso para esta incorporación es el estudio de su viabilidad tecnológica y la verificación de la presencia de sustancias bioactivas en los productos desarrollados.

Capítulo 2: Objetivos

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2.1. HIPÓTESIS DE TRABAJO

Las hipótesis de partida y/o las razones que dan pertinencia a esta investigación son las siguientes:

1) Los extractos vegetales suponen una fuente importante de nutrientes y compuestos bioactivos que podrían aprovechar las industrias agroalimentarias para obtener alimentos funcionales.
2) La composición de los extractos vegetales, especialmente en hidratos de carbono, puede potenciar el crecimiento y multiplicación de bacterias lácticas. En cambio otros componentes (ácidos orgánicos, polifenoles) podrían tener un efecto inhibidor sobre algunas poblaciones de microorganismos.
3) La incorporación de extractos vegetales puede influir en las variables del proceso de elaboración de leches fermentadas (tiempo de fermentación, pH) así como influir en la viabilidad tecnológica del producto final. Igualmente es necesario conocer la aceptación sensorial de los productos desarrollados.
4) También se desconoce el efecto de la presencia de los constituyentes de los extractos vegetales sobre la conservación y vida útil del producto final.
5) Los extractos vegetales poseen actividad antioxidante que potencialmente incorporarán a las leches fermentadas. Asimismo es necesario estudiar el efecto de la interacción proteína-polifenol en las leches fermentadas sobre las propiedades antioxidantes del producto final.
6) La leche contiene ácido linoleico conjugado (CLA) de forma natural. Debido al interés actual que suscita y al potencial de mercado de dicho ácido graso es interesante el estudio del aumento natural de los niveles a través del proceso de fermentación o mediante el uso de precursores.
7) Igualmente se desconoce el efecto del uso de precursores de CLA sobre la conservación y vida útil del producto final.

2.2. OBJETIVO PRINCIPAL

El principal objetivo de la presente Tesis Doctoral fue **desarrollar leches fermentadas saludables** mediante la adición de extractos vegetales ricos en compuestos fenólicos (potencialmente antioxidantes) así como el estudio de las vías de potenciación del contenido en CLA (potencial efecto anti-obesidad).

2.3. OBJETIVOS ESPECÍFICOS

Con la finalidad de alcanzar el objetivo principal se plantearon los siguientes objetivos más concretos:

- Caracterizar desde un punto de vista físico, químico y antioxidante los distintos extractos vegetales.
- Obtener leches fermentadas ricas en los distintos extractos vegetales con el fin de introducir nuevas posibilidades de uso de sub-productos de la industria alimentaria (dátil y membrillo), así como ofrecer un valor añadido (granada).
- Evaluar las principales características de las leches fermentadas enriquecidas tales como sus propiedades químicas, físicas, microbiológicas y sensoriales con el fin de conocer cómo afectan al producto final y su idoneidad.
- Seleccionar la leche fermentada en base a su aceptación sensorial y potencial antioxidante y estudiar el grado de interacción proteína-polifenol.
- Conocer el contenido medio en CLA de leches comerciales de consumo habitual con el fin de estimar los niveles medios ingesta.
- Conocer si el proceso de fermentación de la leche de cabra por los cultivos iniciadores da lugar a un incremento de la concentración inicial de CLA.
- Evaluar la influencia de la fuente de ácido linoleico en el contenido final en CLA en leche fermentada de cabra.
- Evaluar la evolución del contenido en CLA a lo largo de la vida útil comercial de las leches fermentadas de cabra estudiadas.

Capítulo 3: Materiales y Métodos

3.1. INTRODUCCIÓN AL DISEÑO EXPERIMENTAL

En base a los objetivos se estableció un diseño experimental dividido en dos planes de trabajo con la finalidad de estudiar por un lado el uso de extractos vegetales como fuente de compuestos bioactivos en la elaboración de yogur y por otro, evaluar el contenido en ácido linoleico conjugado (CLA) en leches fermentadas y estrategias de potenciación.

- En el **primer bloque experimental** se estudió en una primera fase el uso de dos sub-productos de las industrias del dátil y membrillo (aguas de escaldado) y zumo de granada en la elaboración de yogur enriquecido. En primer lugar se caracterizaron las materias primas en cuanto a su composición fisicoquímica y capacidad antioxidante. Tras ello se procedió a la elaboración de los distintos yogures enriquecidos y a su análisis. Se estudió el efecto de la incorporación de los extractos vegetales sobre la fermentación y las propiedades fisicoquímicas y microbiológicas de los yogures durante su vida útil. La segunda fase se diseñó a partir de los resultados obtenidos en la primera fase. Tal y como se presenta en detalle en la discusión (*Véase* **Discusión general**), en la primera fase se observó que el zumo de granada fue el extracto vegetal que presentó la mayor capacidad antioxidante así como un elevado contenido en compuestos bioactivos. Además el yogur enriquecido en zumo de granada presentó la mayor aceptación por parte de los consumidores. En base a esto se decidió estudiar la partición de los principios bioactivos (compuestos fenólicos) (fase proteica-desproteinizada) así como la capacidad antioxidante del yogur enriquecido.
- El **segundo bloque experimental** se realizó igualmente en dos fases. En primer lugar se efectuó una profunda revisión bibliográfica sobre ingredientes funcionales con potenciales efectos anti-obesidad. De los datos obtenidos se decidió continuar con la investigación en base al CLA por su potencial anti-obesidad e interés actual. Por un lado se evaluó el contenido en CLA en leches fermentadas comerciales de consumo habitual, por otro lado se determinó el contenido en CLA en leche de cabra y el efecto combinado de la adición de

aceite de girasol y cultivos iniciadores en el contenido en CLA de las leches fermentadas obtenidas (convencional/probiótica).

3.2. PROCEDIMIENTO EXPERIMENTAL *A*: EFECTO DE LA INCORPORACIÓN DE EXTRACTOS VEGETALES A LECHES FERMENTADAS TIPO YOGUR

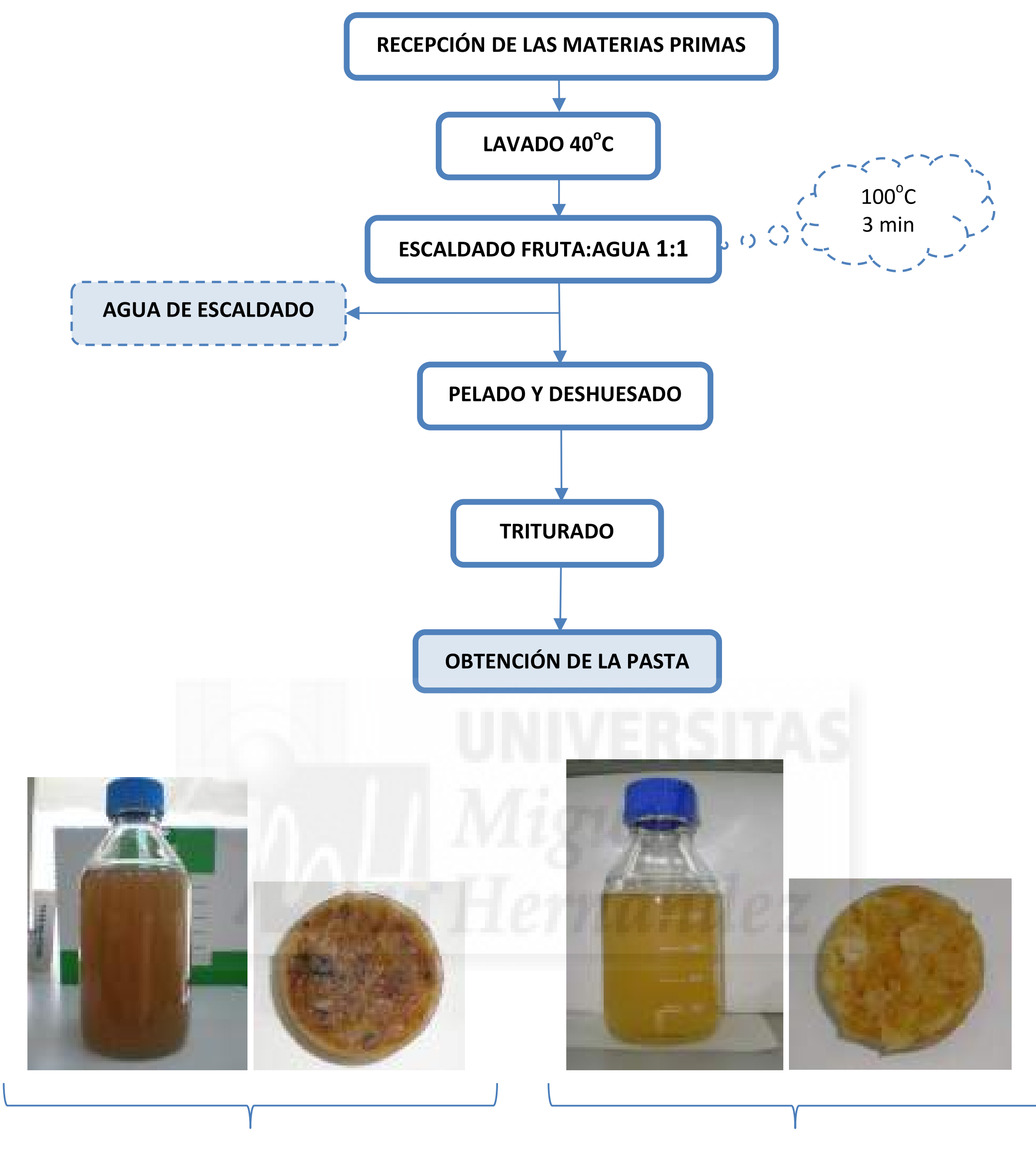
3.2.1. MATERIAL VEGETAL

Los estudios llevados a cabo con **dátiles** se realizaron con dos variedades no comerciales que fueron suministradas por la Estación Phoenix de Elche: Medjoul y Confitera en sus estados 'tamr' (aproximadamente 20% de humedad) y 'rutab' (35-40% de humedad) de maduración, respectivamente.

El **membrillo** fue recolectado del huerto que posee la Universidad Miguel Hernández de Elche (Campus de Desamparados, Orihuela, Alicante).

En la presente Tesis Doctoral se decidió analizar tanto el agua de escaldado de dátil y membrillo (sub-producto líquido) como la pasta resultante de los procesos de pelado, deshuesado y triturado (**Figura 4**).

La **granada**, variedad Mollar de Elche, fue adquirida en un supermercado local. Para la obtención del zumo de arilos la granada se peló manualmente y una vez separados los arilos de la corteza se licuaron y filtraron a través de gasas estériles manteniéndose en congelación hasta su análisis.



AGUA Y PASTA DÁTIL MEDJOUL **AGUA Y PASTA DÁTIL CONFITERA**

Figura 4. Sistema de obtención de la pasta y agua de escaldado de dátil y membrillo.

3.2.2. SISTEMA DE OBTENCIÓN DEL YOGUR ENRIQUECIDO

Para la obtención de yogur con aguas de escaldado de dátil y membrillo se ha optado por la elaboración de un yogur firme con un 15% de leche en polvo. Las etapas

generales de la elaboración de este tipo de yogur son: preparación de la mezcla de materias primas, pasterización, inoculación, fermentación y enfriamiento.

PROCEDIMIENTO:

En el caso del yogur control la leche en polvo se reconstituyó al 15% con agua desionizada. Para la producción de los yogures enriquecidos en agua de escaldado de dátil y membrillo el agua desionizada se sustituyó por el agua de escaldado pasterizada. En las producciones destinadas a análisis sensorial se adicionó un 8% de azúcar. La leche desnatada reconstituida se pasterizó a 80°C durante 30 minutos en baño de agua, tras ello se enfrió hasta alcanzar una temperatura de 45°C. En este punto se adicionó el cultivo estárter (mezcla de termófilos *MY 900* (*EZAL*®): *Streptococcus thermophilus* y *Lactobacillus delbrueckii* subsp. *bulgaricus*) a la dosis recomendada por el fabricante. El mix inoculado se repartió en recipientes estériles y se incubó a 43°C, hasta pH 4.7 y finalmente se refrigeró hasta 4°C.

Para la elaboración de los yogures enriquecidos en zumo de granada se realizaron experiencias preliminares para determinar la máxima incorporación de zumo de arilos que permitiera mantener la estabilidad de la leche fermentada. Se desarrolló el siguiente proceso de elaboración: la leche desnatada en polvo se reconstituyó al 25%; zumo de granada y leche reconstituida fueron pasterizados por separado (para evitar procesos de coagulación) y posteriormente el zumo fue adicionado a la leche hasta una concentración final del 40%. A partir de este punto el proceso es el anteriormente descrito: inoculación, fermentación hasta pH 4.7 (o durante 4 horas) y enfriado.

3.2.3. CARACTERIZACIÓN DE LAS MATERIAS PRIMAS

3.2.3.1. pH y ^{o}Brix

Para la determinación analítica del pH se utilizó un pH-metro modelo 510 (EUTECH INSTRUMENTS, Pte Ltd., Singapore). El nivel de sólidos solubles, expresados como ^{o}Brix, se midió en un refractómetro modelo DR-101 (Coseta S.A., Barcelona, España).

3.2.3.2. Composición proximal

Las siguientes determinaciones únicamente se llevaron a cabo en las muestras de dátil. El contenido en cenizas, proteínas y grasa se determinaron siguiendo Métodos Oficiales (AOAC, 2000). El contenido en humedad se determinó por pérdida de peso tras a calentamiento a 60-65°C en estufa a vacío y peso constante. El contenido en fibra dietética total (TDF) e insoluble (IDF) se determinaron siguiendo el método enzimático-gravimétrico AOAC 991.43. La fibra dietética soluble (SDF) se calculó restando la proporción de IDF a TDF.

3.2.3.3. Determinación de ácidos orgánicos y azúcares

Los ácidos orgánicos y los azúcares se cuantificaron en un cromatógrafo líquido de alta presión (HPLC) Hewlett Packard Serie 1100 (Woldbronn, Alemania). Como fase móvil se utilizó ácido ortofosfórico al 0.1% con un flujo de 0.5 mL/min y la separación de los ácidos y los azúcares se realizó en una columna SUPELCOGEL C-610 H 30 cm de longitud y 7.8 mm de diámetro interno y una precolumna SUPELGUARD C-610 H. La cuantificación se llevó a cabo con un detector de absorbancia a 210 nm DAD G1315A en el caso de los ácidos orgánicos y con detector de índice de refracción RID G-1362A para los azúcares y comparando las áreas de los picos obtenidos en las muestras con las correspondientes a patrones de cada ácido/azúcar de concentración conocida. Las muestras se analizaron a 30°C y el tiempo total del programa de análisis fue de 30 minutos (Doughty, 1995).

3.2.3.4. Determinación del contenido en fenoles y flavonoides totales

Para la realización de dichos análisis y la determinación de la actividad antioxidante se obtuvo el extracto de la pasta de dátil y membrillo siguiendo el método de Al-Farsi y col., (2005). En el caso de las aguas de escaldado, se centrifugaron a 5.000 rpm durante 10 minutos y el sobrenadante fue decantado. En último lugar la extracción de compuestos fenólicos en el zumo de granada se realizó con metanol acidificado (0.003% de ácido clorhídrico concentrado), seguido de una centrifugación (5.000 rpm, 10 min) y concentración a 50°C.

El contenido en fenoles totales (TPC) se determinó utilizando el reactivo de Folin-Ciocalteu’s siguiendo el método de Singleton y Rossi (1965) y los resultados fueron

expresados como mg de equivalentes de ácido gálico (GAE)/L de muestra. Para la determinación del contenido en flavonoides totales (TFC) se siguió el método de Blasa y col., (2005) y los resultados fueron expresados como mg de equivalentes de rutina (RE)/100 g de muestra.

3.2.3.5. Determinación del contenido en antocianos totales

La determinación del contenido en antocianos totales (TAC) se llevó a cabo únicamente en las muestras de granada mediante el método de diferencial de pH descrito por Wrolstad (1993). Los resultados fueron expresados como mg de equivalentes de cianidin-3-glucosido/L de muestra.

3.2.3.6. Determinación de la actividad antioxidante

La determinación de la actividad antioxidante *in vitro* se realizó siguiendo tres métodos analíticos diferentes: (i) secuestro del radical 2,2'-difenil-1-picrilhidrazil (DPPH), siguiendo las recomendaciones de Brand-Williams y col., (1995) donde la cantidad de muestra necesaria para disminuir la absorbancia del DPPH· en un 50% (IC_{50}) se calculó gráficamente; (ii) poder antioxidante de la reducción de hierro (FRAP), según el método descrito por Oyaizu (1986) y estimado en términos de capacidad antioxidante en equivalentes de Trolox (TEAC) en mmol/L; (iii) capacidad quelante del ion ferroso (FIC), según el método utilizado por Singh y Rajini (2004), para la determinación de la concentración necesaria para obtener un 50% de efecto quelante (EC_{50}) se representó el porcentaje de efecto quelante frente a la concentración de muestra.

3.2.4. DETERMINACIONES REALIZADAS EN LOS YOGURES ENRIQUECIDOS

Los yogures fueron muestreados a día 1 de almacenamiento para la determinación de: color, textura y análisis sensorial. Durante los días 1, 7, 14, 21 y 28 de almacenamiento se determinaron: pH, parámetros reológicos, microbiología, ácidos orgánicos, azúcares. Además en los yogures enriquecidos en zumo de granada se determinó a día 1 y 28 de almacenamiento: perfil de compuestos fenólicos, contenido en fenoles, flavonoides y antocianos totales y actividad antioxidante.

3.2.4.1. Determinación del color

Se estudió el espacio de color CIEL*a*b* mediante un colorímetro Minolta CM-2002 (Minolta Camera Co. Osaka, Japan) con aplique para la medición de líquidos CR-A70 (Minolta Camera Co. Osaka, Japan), usando el iluminante D65 y el observador 10°.

3.2.4.2. Determinación de la textura

Para el análisis de la textura del yogur firme se empleó un texturómetro TA-XT2i (Stable Micro Systems, Surrey, England) con una célula de carga de 5 kg. Se realizó un ensayo de penetración para determinar la firmeza del gel.

3.2.4.3. Determinación de los parámetros reológicos

Los parámetros reológicos se determinaron por métodos oscilatorios a 7°C por medio de un reómetro Rheostress 600 (Haake, Karlsruhe, Germany). Las pruebas se realizaron en rodajas de gel de yogur de 1.2 ±0.2 mm de grosor y 35mm. Se empleó geometría plato-plato con platos serrados (35 mm diámetro, 1 mm ranura). Se realizó el test de barrido de frecuencia de 0.01 a 10 Hz a esfuerzo constante de 0.4 Pa, con tres ciclos de medida.

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Figura 5. Instante del corte de la rodaja de yogur.

3.2.4.4. Análisis sensorial

Un total de 36 panelistas, personal y estudiantes de la Universidad Miguel Hernández (Elche) fueron instruidos para la realización de las catas. Los panelistas evaluaron la

apariencia, cremosidad, dulzor, astringencia, acidez, sabor del yogur y aceptación global mediante escala hedónica de 7 puntos (1= Me disgusta en extremo y 7= Me gusta en extremo). En todos los casos un panel de 3 expertos definió los términos a valorar por los panelistas.

3.2.4.5. Determinaciones microbiológicas

Se han realizado determinaciones de los recuentos de estreptococos, lactobacilos y mohos y levaduras durante los días 1, 7, 14, 21 y 28 de almacenamiento de los yogures. Los recuentos de *Streptococcus* spp. se realizaron en agar M-17 (condiciones aeróbicas, 37°C, 24h). Los recuentos de *Lactobacillus* spp. se realizaron sobre agar MRS (condiciones anaeróbicas, 37°C, 48h). Mohos y levaduras fueron enumerados utilizando agar Rosa Bengala (Rose Bengale) con cloranfenicol (condiciones aeróbicas, 28°C, 5 días).

3.2.4.6. Determinación de ácidos orgánicos y azúcares en yogures

Los ácidos orgánicos y azúcares en los yogures se analizaron durante su almacenamiento en refrigeración siguiendo el procedimiento anteriormente descrito (*Véase* apartado ***3.2.3.3. Determinación de ácidos orgánicos y azúcares***).

3.2.4.7. Determinación del grado de interacción proteína-polifenol en yogur con granada

Con el fin de estimar cuantitativamente los compuestos fenólicos que interaccionan con las proteínas del yogur y los que quedan libres en la fase soluble (permeato) se utilizó un sistema a escala de ultrafiltración (Amicon® Ultra-15; Merck Millipore Ltd., Ireland). Mediante este sistema se obtiene una fase soluble desproteinizada, permeato, libre de proteínas de peso molecular superior a 10.000 Da.

3.2.4.8. Determinación del perfil de compuestos fenólicos en yogur con granada

La determinación del perfil de compuestos fenólicos se llevó a cabo únicamente las muestras de granada (zumo, permeato y yogur). Para ello se llevó a cabo una extracción previa de las muestras de yogur siguiendo el método descrito por Karaaslan

y col., (2011). En el caso de las muestras de zumo de granada y permeato se llevó a cabo una extracción con metanol acidificado (*Véase* sección ***3.2.3.4. Determinación del contenido en fenoles y flavonoides totales***).

La identificación de ácido gálico y punicalaginas (α y β) se llevó a cabo en un cromatógrafo líquido de alta presión Hewlett Packard Serie 1200 (Woldbronn, Alemania) acoplado a un detector de red de diodos (HPLC-DAD) según el método de Benavente-García y col., (1999). Los espectros ultravioletas (UV) de los picos individuales se registraron en el rango de 200-600 nm. La cuantificación de los compuestos individuales se llevó a cabo comparando tiempo de retención y espectro con sus correspondientes patrones puros.

La identificación de antocianinas se llevó a cabo utilizando un cromatógrafo líquido ACQUITY de ultra presión (UPLC), detector PDA (Waters Corporation, Milford, MA, USA) y un espectrómetro de micromasa Micromass Q-TOF (Waters, Manchester, UK) equipado con fuente de ionización en electro espray (ESI) operando en modo positivo y negativo. Los análisis se llevaron a cabo con un detector de espectrometría de masas de escaneado completo en un rango espectral de 100-1500 *m/z*. La caracterización de los compuestos individuales se llevó a cabo mediante tiempo de retención y masa molecular.

3.2.4.9. Determinación del contenido en fenoles, flavonoides y antocianos totales en yogur con granada

La determinación del contenido en fenoles y flavonoides totales se llevó a cabo en el yogur de granada y en el permeato de yogur de granada siguiendo protocolos anteriormente descritos (**Véase** sección ***3.2.3.4. Determinación del contenido en fenoles y flavonoides totales***). La determinación de antocianos totales se llevó en los yogures de granada siguiendo el protocolo descrito en la sección ***3.2.3.5. Determinación del contenido en antocianos totales***.

3.2.4.10. Determinación de la actividad antioxidante en yogur con granada

La determinación de la actividad antioxidante *in vitro* se realizó únicamente en los yogures de granada siguiendo dos métodos analíticos diferentes: (i) secuestro del

radical 2,2'-difenil-1-picrilhidrazil (DPPH), siguiendo las recomendaciones de Brand-Williams y col., (1995), donde la cantidad de muestra necesaria para disminuir la absorbancia del DPPH· en un 50% (IC_{50}) se calculó gráficamente y (ii) poder antioxidante de la reducción de hierro (FRAP), según el método descrito por Oyaizu (1986) y estimado en términos de capacidad antioxidante en equivalentes de Trolox (TEAC) en mmol/L .

3.3. PROCEDIMIENTO EXPERIMENTAL *B*: ÁCIDOS GRASOS CONJUGADOS COMO INGREDIENTES FUNCIONALES Y EVALUACIÓN DEL CONTENIDO EN ÁCIDO LINOLEICO CONJUGADO (CLA) EN LECHES FERMENTADAS

3.3.1. PROCESO DE REVISIÓN BIBLIOGRÁFICA

La revisión bibliográfica se ha efectuado utilizando las bases de datos Science Direct (1990 a 2012) y SCOPUS (1990 a 2012) existentes en la Universidad Miguel Hernández de Elche. Esta revisión se basó, estrictamente, en las relaciones de los ácidos grasos con su efecto anti-obesidad, de forma científicamente demostrada y publicada en revistas de reconocido prestigio internacional.

En la **Tabla 12** se puede apreciar el número de citas bibliográficas, relacionadas con esta revisión, de las tres bases de datos consultadas.

Tabla 12. Número de citas bibliográficas relacionadas con ácidos grasos y efecto anti-obesidad, realizadas en las bases de datos Science Direct y SCOPUS.

BASE DE DATOS	Science Direct	SCOPUS
Ácidos grasos	374.792	211.307
Ácidos grasos y anti-obesidad	23.652	442
CLA	10.665	4.454
CLA y anti-obesidad	1.170	67

Abreviaciones: CLA, ácido linoleico conjugado.

Se han estudiado, durante el desarrollo de este trabajo de revisión, un total de 900 referencias de revistas de reconocido prestigio internacional relacionadas, todas ellas, con el tema de investigación: ‘ingredientes alimentarios como agentes anti-obesidad’. La composición final del trabajo de revisión se dividió en las siguientes secciones:

- → Ácidos grasos bioactivos.
- → Compuestos fenólicos.
- → Soja.
- → Esteroles.
- → Calcio dietético.
- → Fibra dietética.

3.3.2. CLA EN LECHES FERMENTADAS COMERCIALES

3.3.2.1. Selección de muestras comerciales

Un total de 24 leches fermentadas comerciales (de leche de vaca) fueron adquiridas por triplicado de diferentes supermercados locales. Según la información suministrada en el etiquetado se muestrearon las siguientes combinaciones en función del contenido graso y cultivo iniciador (tradicional/probiótico):

Tabla 13. Combinaciones de leches comerciales analizadas en función del cultivo estárter y el contenido en grasa.

Contenido en grasa	Cultivo estárter		
	CTY[1]	CTY + *Bifidobacterium* spp.	CTY + *L. casei*
> 8%	4	---	---
1.4% - 4%	5	3	3
<0.5%	3	3	3

[1]CTY: cultivos tradicionales del yogur (*L. delbrueckii* subsp. *bulgaricus* y *S. thermophilus*).

3.3.2.2. *Análisis de ácidos grasos por cromatografía de gases acoplada a detector de ionización de llama (GC-FID)*

Los ácidos grasos fueron metilados *in situ* según el método descrito por Park y Goins (1994). Los ésteres metílicos de los ácidos grasos (FAMEs) se analizaron en un cromatógrafo de gases Agilent (modelo 6890, Palo Alto, CA, USA) equipado con un detector de ionización de llama (FID) y una columna capilar DB-23 (30 m largo, 0.25 µm de espesor de película, 0.25 mm diámetro interno; J&W Scientific, Agilent Technologies). Los FAMEs fueron identificados por comparación con los tiempos de retención de los estándares de FAMEs y cuantificados a partir de área y su comparación con el área del patrón interno correspondiente (C9:0 y C17:0).

3.3.3. CLA EN LECHES FERMENTADAS DE CABRA

3.3.3.1. Materias primas

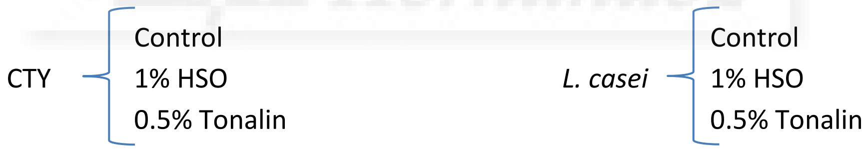
La **leche de cabra** fue recolectada en la granja de la Universidad Miguel Hernández (Orihuela, Alicante). El **aceite de girasol hidrolizado** (HSO) se preparó según el método descrito por Xu y col., (2004) adicionándose a una concentración del 1% en producto final. Con el fin de confirmar los resultados se elaboró un lote de yogures con un 0.5% de Tonalin® (80% CLA) en producto final. Se estudiaron el efecto de dos **cultivos estárter**: (1) cultivos tradicional del yogur (CTY) (ratio 1:1 de *L. delbrueckii* subsp. *bulgaricus* y *S. thermophilus*, YF-L811 Yo-Flex®) y (2) *L. casei* (LC-01 nu-trish®), suministrados por Chr. Hansen (Hørsholm, Denmark) a la dosis recomendada por el proveedor.

3.3.3.2. Análisis químico de la leche de cabra

La composición química de la leche de cabra se determinó automáticamente en Milko Scan FT 120 (Foss Elctric, Hillerød, Denmark). El pH se determinó en un pH-metro modelo 510 (EUTECH INSTRUMENTS, Pte Ltd., Singapore).

3.3.3.3. Sistema de obtención de las leches de cabra fermentadas

Tras la pasterización de la leche cruda de cabra, se elaboraron los siguientes lotes de leches fermentadas:



Para la obtención de las leches fermentadas con CTY se siguió el procedimiento descrito en la sección ***3.2.2. Sistema de obtención del yogur enriquecido***. Para la obtención de la leche fermentada con *L. casei* el procedimiento a seguir es el mismo excepto en la temperatura de incubación (37°C) y el pH alcanzado al final de la fermentación (3.7).

En los 0 (punto final de la fermentación), 1, 14, 28 y 35 de almacenamiento se analizaron muestras para determinar perfil de ácidos grasos totales, microbiología y pH.

3.3.3.4. Determinación del grado de hidrólisis del aceite de girasol

Los productos de reacción de la hidrólisis del aceite de girasol se determinaron por cromatografía gaseosa (Luna y col., 2013) utilizado un cromatógrafo de gases HP 5890 Series II, conectado a una columna capilar HT5 (25 m x 0.32 mm I.D x 0.1 µm, SGE, Supelco) con un detector de Ionización por llama (FID) a 450ºC e inyección splitless a 350ºC. Este método consiste básicamente en una modificación e integración de dos métodos oficiales, UNE EN ISO 14103 (ésteres) y UNE EN ISO 14105 (glicéridos), usando como patrón interno: cetano (> 99.9 % Sigma Aldrich), para cuantificar el contenido de glicerol, ésteres etílicos y glicéridos (-mono, -di y triglicéridos), respectivamente (Calero y col., 2014).

3.3.3.5. Análisis de ácidos grasos por cromatografía de gases acoplada a detector de ionización de llama (GC-FID)

La composición en ácidos grasos de los FAMEs en leche de cabra y las respectivas leches fermentadas se determinó siguiendo el protocolo anteriormente descrito en la sección ***3.3.2.2. Análisis de ácidos grasos por cromatografía de gases acoplada a detector de ionización de llama (GC-FID)***.

3.3.3.6. Determinaciones microbiológicas en leches fermentadas

Se han realizado determinaciones de los recuentos de estreptococos y lactobacilos en las leches fermentadas. El recuento de *Streptococcus* spp. se realizó en agar M-17 y el de *Lactobacillus* spp. sobre agar MRS, bajo las condiciones descritas en la sección ***3.2.4.5. Determinaciones microbiológicas***.

3.4. ANÁLISIS ESTADÍSTICO

Los datos han sido analizados mediante el programa estadístico *Statistical Package for the Social Sciences* (SPSS v20.0) para Windows (SPSS Inc., Chicago, IL, EEUU). Cualquier diferencia estadísticamente significativa entre tratamientos ha sido determinada mediante análisis de la varianza (ANOVA). La naturaleza exacta de las diferencias entre grupos fue determinada mediante la prueba de Tukey's parwaise comparisions post-hoc test. En todos los casos las diferencias fueron consideradas significativas para

p<0,05. En general, los datos a lo largo de la presente Tesis Doctoral se expresan como medias y error estándar de la media. Solo en el artículo 'Conjugated linoleic acid content in fermented goat milk as affected by the starter culture and the presence of free linoleic acid' se utilizó el programa R software (R Core Team 2013, URL http://www.R-project.org/) y se ejecutó un análisis de modelos mixtos para el tratamiento de los datos.

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Capítulo 4: Discusión General

4.1. INTRODUCCIÓN A LA DISCUSIÓN

El compuesto (funcional/bioactivo) ideal para la fortificación de alimentos sería aquél que llegue al alimento en sus mayores niveles de biodisponibilidad, que no disminuya el valor nutricional del vehículo alimentario, que no altere sus propiedades sensoriales, que pueda ser usado para fortificar tanto alimentos líquidos como sólidos, que sea resistente al procesado y por último, en lo posible que sea de bajo coste (Gaucheron, 2000).

En base a esto, en el presente estudio se evaluó el potencial de dos sub-productos de la industria del dátil y membrillo, y del zumo de granada como fuente de compuestos bioactivos (ácidos orgánicos, azúcares, fibra, compuestos fenólicos) y el potencial del ácido linoleico conjugado como ingrediente con propiedades beneficiosas frente a enfermedades cardiovasculares y posible efecto anti-obesidad, como compuestos para la fortificación de leche y productos lácteos.

Este capítulo recoge los principales resultados y una discusión de los diferentes trabajos realizados. Las versiones completas de los mismos se encuentran en los correspondientes artículos publicados o en proceso de revisión en revistas internacionales incluidas en el *Journal Citations Reports* y se adjuntan al final de esta memoria.

4.2. BLOQUE I: EFECTO DE LA INCORPORACIÓN DE EXTRACTOS VEGETALES A LECHES FERMENTADAS TIPO YOGUR

4.2.1. CARACTERIZACIÓN DEL MATERIAL VEGETAL

Los dátiles sujetos al presente estudio se caracterizan por ser no comerciales, descartados durante su industrialización. Los dátiles Medjoul fueron descartados tras el destrío debido a que no cumplían con los criterios de calidad post-cosecha (dátiles rotos, con insuficiente tamaño o color, con apariencia poco atractiva). Los dátiles Confitera necesitan de altas temperaturas durante su maduración y las condiciones climatológicas del palmeral de Elche no son las óptimas, con lo que habitualmente estos dátiles se recogen prematuramente para evitar que el fruto se agriete y pueda sufrir posterior contaminación. Se hace necesario pues caracterizar estos cultivares no comerciales de la industria del dátil española para dar salida a sus potenciales usos en la industria alimentaria.

En cuanto al membrillo, Murcia, una región próxima a las instalaciones de nuestro campus universitario, es una de las zonas más productoras a nivel nacional. Previos estudios han mostrado el potencial efecto beneficioso de las diversas partes del fruto (*Véase* sección **1.6.2. Membrillo**) si bien en la presente tesis doctoral nos centraremos en investigar el posible uso del agua de escaldado resultante del procesado del fruto, así como la pasta obtenida pues consideramos que es necesaria su caracterización como materia prima principal.

El estudio del zumo de granada como ingrediente en la fortificación de yogures tiene su explicación por el interés que ha suscitado dicha fruta en los últimos años, en especial por su potencial antioxidante. Los yogures con frutas son uno de los productos lácteos con mayor expectativa de venta, y mucho más si son 'bajos en grasas'.

Los resultados de la caracterización de la pasta y agua de escaldado obtenidas tras el procesado de los dátiles se presentan en dos artículos, uno de ellos publicado en la revista *Food Science and Technology* (2014, 2(3): 34-40) y el otro publicado en la revista *Food and Bioproducts Processing* (2012, 90: 506-514); la caracterización de la pasta y agua de escaldado de membrillo se encuentra publicada en la revista *LWT* -

Food Science and Technology (2011, 44: 1388-1395) y la caracterización del zumo de granada se presenta en dos artículos, uno de ellos publicado en el *Journal of Agricultural and Food Chemistry* (DOI: 10.1021/jf501503h) y el otro publicado en la revista *Milchwissenschaft* (2012, 67(2): 177-180).

4.2.1.1. Composición proximal de los dátiles

En la **Tabla 14** se presenta la composición media de las pastas y agua de escaldado de los dos cultivares de dátiles. La pasta de dátil tiene una elevada tendencia a retener agua (Martín-Sánchez y col., 2014) y ello se ve reflejado en las pastas en estudio, de hecho el contenido en humedad de la carne de dátil Medjoul (sin escaldar) fue de 34.73% y de 59.18% en el caso de Confitera. La pasta de dátil Confitera (CP) al encontrarse en un estadío de maduración más fresco presentó una mayor humedad, además está demostrado que el escaldado captura una importante cantidad de agua (Martín-Sánchez y col., 2014). El contenido en proteína, cenizas, fibra dietética (total, soluble e insoluble) fue mayor en la pasta de dátil Medjoul (MP) que en CP, aunque todos los valores encontrados están en concordancia con resultados publicados en otros estudios (Baraem y col., 2006). Por otro lado puede considerarse que las pastas de dátiles en estudio tienen un alto contenido en fibra pues se han encontrado valores comprendidos entre 6.4-11.5% (Al-Shahib y Marshall, 2003) y 8.1-12.3% (Martín-Sánchez y col., 2013) en otros estudios. En cuanto a las aguas de escaldado, la de Medjoul (MBW) presentó mayor contenido en proteína y cenizas que el agua de escaldado Confitera (CBW).

Es interesante destacar que el escaldado mejora ciertas propiedades tecnológicas de los dátiles. La capacidad de retención de agua, la capacidad de retención de aceite y la estabilidad de la emulsión se ven aumentadas, propiedades que son muy importantes en la obtención de una textura adecuada en ciertos productos (Martín-Sánchez y col., 2013; 2014).

Tabla 14. Contenido en humedad, proteína, grasa, cenizas, fibra dietética total (TDF), fibra dietética insoluble (IDF) y fibra dietética soluble (SDF) de las pastas y aguas de escaldado de los dátiles Medjoul y Confitera (media ± error estándar).

	MP	MBW	CP	CBW
Humedad (g/100 g)	60.2 ± 0.47^{a}	93.86 ± 0.12^{A}	70.27 ± 0.27^{b}	97.97 ± 0.17^{B}
Proteína* (g/100 g)	3.51 ± 0.10^{b}	0.71 ± 0.09^{B}	2.62 ± 0.11^{a}	0.19 ± 0.07^{A}
Grasa* (g/100 g)	0.17 ± 0.04^{b}	---	0.13 ± 0.05^{a}	---
Cenizas* (g/100 g)	3.32 ± 0.12^{b}	0.67 ± 0.07^{B}	2.48 ± 0.10^{a}	0.18 ± 0.02^{A}
TDF* (g/100 g)	12.43 ± 0.18^{b}	n.d.	9.29 ± 0.08^{a}	n.d.
IDF* (g/100 g)	10.22 ± 0.46^{b}	n.d.	7.63 ± 0.22^{a}	n.d.
SDF* (g/100 g)	2.21 ± 0.14^{b}	n.d.	1.64 ± 0.05^{a}	n.d.

*Los valores se presentan en base seca.
Abreviaciones: MP, pasta de dátil Medjoul; MBW, agua de escaldado de dátil Medjoul; CP, pasta de dátil Confitera; CBW, agua de escaldado de dátil Confitera; n.d., no determinado.
Valores en la misma fila con distinta letra minúscula difieren significativamente ($p<0.05$) entre pastas. Valores en la misma fila con distinta letra mayúscula difieren significativamente ($p<0.05$) entre aguas de escaldado.

4.2.1.2. Contenido en azúcares

Las materias primas con mayor contenido en azúcares fueron los dátiles ($p<0.05$), tanto en las pastas como en las aguas de escaldado (**Tablas 15 y 16**) y particularmente MP con un contenido en azúcares de 33.02 g/100 g. Al-Farsi y Lee (2008) revisaron los constituyentes nutricionales de unas 80 referencias de dátiles siendo fructosa, glucosa y sacarosa los únicos azúcares detectados en dátiles frescos y secos, con un conteniendo medio en dátiles frescos de 19.4, 22.8 y 4.03 g/100 g respectivamente, dicho contenido aumenta cuando el dátil es seco. Además fructosa y glucosa están presentes en cantidades similares (Al-Farsi y Lee, 2008) y como se puede observar esto se cumple tanto en las pastas como en las aguas de escaldado (**Tabla 15**). Confitera es un cultivar con un contenido relativamente bajo en azúcares en comparación con otros cultivares de dátiles debido a la falta de calor durante su maduración y a su pronta recolección.

Los principales azúcares detectados en las muestras de membrillo fueron fructosa, manitol y glucosa. Rodríguez-Guisado y col., (2009) encontraron niveles algo superiores de fructosa y glucosa (7.95 y 5.00 g/100 g, respectivamente) en membrillos cultivados en el sudeste español. Aunque el membrillo presenta un contenido

relativamente alto en azúcares, su consumo en fresco y aceptación por parte de los consumidores es comparativamente bajo debido a su acidez, amargor y astringencia. Por ello y para propósitos industriales es importante que el contenido en azúcares del fruto sea alto.

Tabla 15. Contenido en azúcares (g/100 g) de las distintas materias primas (media ± error estándar).

	Sacarosa	Glucosa	Fructosa	Manitol	Lactosa	AZÚCARES TOTALES
LDR	n.d.	n.d.	n.d.	n.d.	6.40±0.65	6.40±0.65^{b,c}
MP	0.75±0.10^{b,c}	17.00±0.15^{e}	15.27±0.13^{e}	n.d.	n.d.	33.02±0.18^{e}
MBW	0.71±0.22^{b,c}	2.90±0.62^{c}	2.53±0.56^{b}	n.d.	n.d.	6.14±1.39^{b,c}
CP	1.69±0.11^{d}	10.99±0.67^{d}	9.85±0.62^{d}	n.d.	n.d.	22.53±1.40^{d}
CBW	0.86±0.08^{c}	0.18±0.01^{a,b}	0.17±0.00^{a}	n.d.	n.d.	1.20±0.08^{a}
QP	0.23±0.00^{a,b}	2.12±0.08^{b,c}	5.09±0.18^{c}	2.23±0.07^{b}	n.d.	9.67±0.33^{c}
QBW	0.06±0.01^{a}	0.60±0.15^{a,b}	1.42±0.36^{a,b}	0.62±0.15^{a}	n.d.	2.70±0.67^{a,b}
PGJ	0.48±0.09	6.67±0.80	7.27±0.42	n.d.	n.d.	14.42±1.31

Abreviaciones: LDR, leche desnatada reconstituida; MP, pasta de dátil Medjoul; MBW, agua de escaldado de dátil Medjoul; CP, pasta de dátil Confitera; CBW, agua de escaldado de dátil Confitera; QP, pasta de membrillo; QBW, agua de escaldado de membrillo; PGJ, zumo de granada; n.d., no detectado. Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

El zumo de granada es rico en fructosa y glucosa, con un contenido minoritario en sacarosa. El hecho de que tanto en membrillo como en zumo de granada el contenido en fructosa sea superior al de glucosa puede ser ventajoso pues la fructosa es aproximadamente el doble de dulce que la glucosa (Levin y col., 2000). El contenido en azúcares del PGJ es similar al publicado por Hasnaoui y col., (2011) y Melgarejo y col., (2000) y difiere en cuanto a la prevalencia de azúcares respecto al estudio de Ozgen y col., (2008).

Para un mejor entendimiento de los resultados que se presentarán en cuanto a la caracterización de los yogures enriquecidos se caracterizó la leche desnatada reconstituida como materia prima y como era de esperar el azúcar mayoritario detectado fue la lactosa.

En cuanto a su pH (**Tabla 16**), el hecho de que los dátiles Medjoul tengan un pH cercano a la neutralidad hace que sean candidatos ideales para ser incorporados a

otros productos. Tanto QP como PGJ presentaban pH mucho más ácidos, lo cual se debe tener en cuenta pues puede modificar la acidez del producto a enriquecer.

Tabla 16. Contenido en solidos solubles totales (°Brix) y pH de las distintas materias primas.

	pH	°Brix
MP	6.98 ± 0.01	39.80 ± 0.58
MBW	6.25 ± 0.03	8.00 ± 0.10
CP	5.80 ± 0.00	29.73 ± 0.07
CBW	5.70 ± 0.00	2.17 ± 0.05
QP	3.92 ± 0.00	11.73 ± 0.08
QBW	4.83 ± 0.01	3.53 ± 0.03
PGJ	4.09 ± 0.00	14.33 ± 0.02

Abreviaciones: LDR, leche desnatada reconstituida; MP, pasta de dátil Medjoul; MBW, agua de escaldado de dátil Medjoul; CP, pasta de dátil Confitera; CBW, agua de escaldado de dátil Confitera; QP, pasta de membrillo; QBW, agua de escaldado de membrillo; PGJ, zumo de granada; n.d., no detectado. Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

En general el aporte en sólidos solubles de las distintas materias primas se traducirá en una fortificación en el contenido en solidos totales en los yogures enriquecidos.

4.2.1.3. Contenido en ácidos orgánicos

La **Tabla 17** muestra el perfil de ácidos orgánicos de las distintas materias primas. Las pastas de dátil Medjoul y de membrillo presentaron la mayor acidez total. En MP el ácido málico fue el mayoritario, este mismo comportamiento se vio en otros dátiles estudiados (Al-Farsi y col., 2005; Amorós y col., 2009). En cambio tanto en CP, CBW y MBW el ácido orgánico predominante fue el succínico. Desde un punto de vista tecnológico que el ácido málico sea el predominante es considerado positivo pues el ácido málico actúa como potenciador del flavor (Martín-Sánchez y col., 2014). Existe controversia entre estudios llevados a cabo en dátiles en cuanto a su composición en ácidos orgánicos. Muchos autores apuntan a que el contenido en ácido málico disminuye conforme el fruto madura (Rastegar y col., (2012) mientras que otros autores respaldan lo opuesto (Amorós y col., 2009, Martín-Sánchez y col., 2014). En ciertos estudios se ha mostrado que en estados más inmaduros (Khalal) el ácido succínico fue el mayoritario (Mortazavi y col., 2010). También es esperable que la acidez total disminuya conforme aumenta el estado de maduración pero en el

presente estudio no podemos caer en el error de comparar entre estados de maduración pues no se trata de comparaciones entre el mismo cultivar. En las pastas de dátil otros ácidos orgánicos como el acético, fumárico y cítrico estuvieron presentes en menor concentración. El ácido ascórbico sólo se detectó en las pastas, probablemente debido a su inactivación térmica durante el escaldado. Durante el escaldado la parte interna del fruto es capaz de retener dicho ácido mientras que en el agua alcanza temperaturas superiores durante un tiempo más prolongado con lo que el ácido ascórbico es completamente inactivado. En ambas aguas de escaldado el ácido succínico fue el mayoritario seguido del acético.

Ambas pastas de dátil presentaron una acidez similar, sin embargo entre las aguas de escaldado CBW presentó una mayor acidez total de modo que el escaldado afectó más al cultivar Confitera de modo que una gran parte de los ácidos orgánicos solubilizaron y pasaron al agua.

El ácido orgánico mayoritario en la pasta de membrillo fue el acético, además fórmico, málico y succínico en menores concentraciones. En QBW de nuevo acético fue el mayoritario junto con málico en similar concentración. El elevado contenido en ácido acético es señal de una sobre maduración del fruto; como se ha justificado a lo largo de la presente Tesis uno de los objetivos es la valorización de frutos que por diversas causas no pueden ser suministrados para su consumo en fresco (como en el caso de los cultivares de dátiles), en este sentido los membrillos se seleccionaron en avanzado estado de maduración para evaluar su posible revalorización. Otros autores han encontrado el ácido málico como mayoritario (Rodríguez-Guisado y col., 2009; Silva y col., 2004b) mientras que un reciente estudio ha mostrado el ácido fítico (no detectado en el presente estudio) como el más abundante de manera significativa (Szychowski y col., 2014). Las diferencias encontradas pueden atribuirse a la técnica de extracción empleada, al cultivar/clon, al estado de maduración en el que se recolectó el fruto, entre otras (Szychowski y col., 2014).

Tabla 17. Contenido en ácidos orgánicos (mg/100 g) y acidez total (%) de las distintas materias primas (media ± error estándar).

Ácido orgánico	LDR	MP	MBW	CP	CBW	QP	QBW	PGJ
Oxálico	3.60±0.44[a]	53.29±2.51[c]	0.22±0.03[a]	41.41±2.94[b]	4.29±0.11[a]	n.d.	n.d.	3.96±0.15[a]
Cítrico	233.41±24.30[d]	155.43±3.97[c]	9.72±0.83[a]	159.93±12.46[c]	70.27±2.06[b]	84.72±2.73[b]	34.41±5.42[a,b]	262.11±40.88[d]
Málico	2.47±0.69[a]	764.58±0.38[d]	15.78±0.76[a]	579.01±43.07[c]	22.22±0.21[a]	303.73±12.63[b]	97.81±28.13[a]	748.80±5.31[d]
Ascórbico	0.37±0.11[a]	12.64±1.54[b]	n.d.	11.14±1.09[b]	n.d.	10.40±0.11[b]	2.89±1.14[a]	87.49±10.04[c]
Succínico	n.d.	507.79±9.19	142.28±21.61	640.19±53.07	276.00±17.20	270.14±22.16	63.69±24.42	n.d.
Láctico	122.23±3.93	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fórmico	n.d.	111.68±21.55[a,b]	5.71±0.18[a]	129.31±19.83[b]	52.40±2.31[a,b]	318.97±39.89[c]	53.92±22.09[a,b]	n.d.
Acético	14.85±1.87[a,b]	179.15±25.54[a,b]	33.50±5.01[a]	244.08±37.14[b]	198.74±1.76[a,b]	684.60±49.29[c]	103.09±34.24[a,b]	n.d.
Fumárico	3.36±0.12[a]	187.45±0.33[d]	9.18±1.32[a]	61.33±5.85[c]	52.44±3.07[b]	11.01±0.99[a]	1.54±1.54[a]	9.80±2.04[a]
ACIDEZ TOTAL	0.38±0.07[b]	1.97±0.05[c]	0.22±0.03[a]	1.87±0.18[c]	0.68±0.04[a,b]	1.68±0.12[c]	0.36±0.12[a,b]	1.11±0.03[b]

Abreviaciones: LDR, leche desnatada reconstituida; MP, pasta de dátil Medjoul; MBW, agua de escaldado de dátil Medjoul; CP, pasta de dátil Confitera; CBW, agua de escaldado de dátil Confitera; QP, pasta de membrillo; QBW, agua de escaldado de membrillo; PGJ, zumo de granada; n.d., no detectado.
Valores en la misma fila con distinta letra difieren significativamente ($p<0.05$).

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En el zumo de granada (PGJ) el ácido málico fue el mayoritario seguido de cítrico y ascórbico, otros ácidos como fumárico y oxálico se detectaron en menor concentración. Se han encontrado diferencias en relación a la literatura revisada; estudios han detectado cantidades superiores de dichos ácidos (Hasnaoui y col., 2011) mientras que en otras investigaciones dichas cantidades fueron inferiores (Melgarejo y col., 2000; Ozgen y col., 2008).

Al igual que en el estudio de Tormo e Izco (2004) el principal ácido orgánico identificado en la leche desnatada fue el ácido cítrico (se asume que el citrato es incluido en la detección) seguido de ácido láctico y otros ácidos orgánicos (oxálico, fumárico, málico y ascórbico) en menor concentración. Como Tormo e Izco (2004) explicaron es posible que parte del citrato presente en el suero estuviera en forma insoluble en la muestra una vez disuelta en agua y que sin embargo una vez que la muestra es inyectada y mezclada con la fase ácida todo el citrato quedara completamente disuelto para su detección.

En todas las materias primas analizadas se detectó un gran pico en el tiempo de retención del ácido tartárico, pero no ha sido incluido en la **Tabla 17** pues existen dudas razonables sobre su verdadera identificación. Este fenómeno de picos fantasma puede darse en los métodos de análisis en que la identificación se realiza solo en base a los tiempos de retención, los espectros de dichos picos pueden aclarar la identificación pero persiste una probabilidad de confusión.

4.2.1.4. Contenido en fenoles y flavonoides totales

Los compuestos fenólicos han atraído considerablemente la atención de los investigadores principalmente por el papel que juegan como antioxidantes, si bien asimismo contribuyen a los atributos sensoriales de los frutos (amargor y astringencia) (Hamazuzu y col., 2006). En la **Tabla 18** se presenta el contenido en fenoles y flavonoides totales de las distintas materias primas. Según Huang y col., (2005) el contenido en fenoles totales (TPC) determinado por el reactivo de Folin-Ciocalteu puede ser utilizado para estimar la capacidad reductora de un antioxidante. La pasta de membrillo presentó el mayor contenido en fenoles y flavonoides totales ($p<0.05$), con lo que se trata de la materia prima con mayor capacidad de disminuir o prevenir la

oxidación de otras moléculas. Recientemente Wojdyło y col., (2013) caracterizaron el perfil fitoquímico de diferentes variedades y genotipos de membrillo demostrando poseer una elevada actividad biológica, especialmente debida a compuestos pertenecientes a la clase flavan-3-ols y derivados del ácido clorogénico. Además la relación procianidinas/flavan-3-ols es el contribuyente más importante a su demostrada actividad antioxidante (Wojdyło y col., 2013). Extractos de membrillo han mostrado tener un contenido en compuestos fenólicos superior al de la manzana y pera (Fattouch y col., 2008). Es interesante destacar que el contenido en compuestos fenólicos no varía durante el procesado de membrillo para la elaboración de mermelada, con lo que los compuestos fenólicos presentes son bastante estables a la aplicación de tratamientos tecnológicos. Entre los dos cultivares de dátiles Confitera es el que contiene la mayor cantidad de fenoles y flavonoides; además CBW puede considerarse como fuente de compuestos fenólicos pues su contenido fue similar al de la pasta.

La presencia de compuestos fenólicos en las aguas de escaldado puede deberse a procesos de lixiviación durante el escaldado. Entre las aguas de escaldado CBW presentó la mayor concentración en fenoles totales y QBW en flavonoides totales. En dátiles el secado natural que se da durante su maduración es considerado como desfavorable para los antioxidantes naturales, como fenoles y flavonoides, debido a la posibilidad de descomposición oxidativa vía enzimática (polifenol oxidasa (PPO) y glucosidasa) o bien por degradación térmica (Al-Farsi y Lee, 2008). Por otro lado, Martín-Sánchez y col., (2014) observaron un notable aumento en el contenido en fenoles totales tras el escaldado de dátiles posiblemente debido a la inactivación de PPO durante el escaldado.

El zumo de granada presentó niveles superiores de fenoles totales que otros zumos de frutas naturales como la naranja, uva tinta, uva blanca, mango y melocotón (Mahdavi y col., 2010). En el estudio posterior realizado por nuestro grupo de investigación "Antioxidant activity and interactions protein-polyphenol in a pomegranate (*Punica granatum* L.) yogurt" e incluido en la presente Tesis se obtuvieron valores inferiores de fenoles totales (707.25 mg GAE/L) y flavonoides totales (75.50 mg RE/100g) para el mismo cultivar de granada. Tanto la composición de los arilos como las diferentes

propiedades texturales de los tejidos del fruto pueden influir la transferencia de compuestos fenólicos durante el procesado a zumo (Fischer y col., 2013). Atendiendo a los resultados presentados en la **Tabla 18** el contenido en fenoles es similar al encontrado en otros zumos de granada por Ozgen y col., (2008) (1507 mg GAE/L) e inferiores a los obtenidos por Mahdavi y col., (2010) (4214.20 mg GAE/L) y Tehranifara y col., 2010) (2957.9–9853.7 mg GAE/L) aunque dichas estas variaciones pueden deberse a diferencias entre cultivares, prácticas agrícolas, método de determinación usado, entre otros (Çam y col., 2009). En el fruto de la granada la concentración máxima de flavonoides totales se encuentra en la piel seguido de la pulpa (albedo) (Li y col., 2006).

Tabla 18. Contenido en fenoles totales (TPC) y flavonoides totales (TFC) de las distintas materias primas (media ± error estándar).

	TPC (mg GAE/L)	TFC (mg RE/100g)
MP	543.58 ± 9.39	57.70 ± 0.72
MBW	200.57 ± 2.86	27.88 ± 0.79
CP	621.92 ± 7.72	73.61 ± 0.85
CBW	521.05 ± 8.57	65.94 ± 2.60
QP	1595.67 ± 7.27	459.86 ± 23.21
QBW	410.80 ± 15.88	70.40 ± 2.90
PGJ	1406.83 ± 0.79	80.21 ± 3.54

Abreviaciones: GAE, equivalentes de ácido gálico; RE, equivalentes de rutina; MP, pasta de dátil Medjoul; MBW, agua de escaldado de dátil Medjoul; CP, pasta de dátil Confitera; CBW, agua de escaldado de dátil Confitera; QP, pasta de membrillo; QBW, agua de escaldado de membrillo; PGJ, zumo de granada.
Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

4.2.1.5. Contenido en antocianos totales (yogur con granada)

El contenido en antocianos totales del zumo de granada fue de 126.04 mg de equivalentes de cianidín-3-glucosido (CGE)/L. Cuantitativamente nuestros resultados fueron superiores a los valores medios publicados por Elfalleh y col., (2011) (39.19 mg CGE/L) y Ozgen y col., (2008) (60.0 mg CGE/L) para zumos procedentes de arilos, aunque este último presentando una gran variabilidad. Según Proteggente y col., (2002) las frutas y verduras ricas en antocianos son las que poseen la mayor actividad antioxidante. El conocimiento sobre la capacidad antioxidante de los alimentos sería interesante y de gran utilidad en estudios epidemiológicos (Proteggente y col., 2002).

4.2.1.6. Actividad antioxidante

Para la evaluación de la actividad antioxidante (AA) *in vitro* se deben de ensayar una mezcla de métodos para cubrir todos los aspectos relacionados con la eficacia antioxidante pues la AA medida a través de un ensayo individual refleja sólo la reactividad química bajo las condiciones específicas aplicadas en dicho ensayo (Huang y col., 2005). En este sentido se han evaluado tres ensayos basados en métodos espectrofotométricos para evaluar la AA de las distintas materias primas (**Tabla 19**).

La capacidad de secuestrar radicales de las distintas materias primas se analizó utilizando el radical libre DPPH. La Tabla X muestra las concentraciones que se requieren para secuestrar al radical DPPH y los valores de secuestro como % de inhibición. De entre todas las muestras analizadas, el zumo de granada fue con diferencia la que presentó la mayor capacidad de secuestrar radicales a una concentración de 10 g/100g. Esto quiere decir que es capaz de prevenir reacciones en cadena de iniciación y propagación de radicales libres (Viuda-Martos y col., 2011). En el análisis del DPPH·, la mayor AA se refleja en el menor IC_{50}; en este sentido el orden de AA fue: PGJ>QP>QBW>MP>MBW>CBW>CP. El zumo de granada presentó mayor actividad secuestrante de radicales (menor IC_{50}) que algunos co-productos de la industria transformadora de zumo de granada (IC_{50}= 11.6-37.8) (Viuda-Martos y col., 2011) los cuales contenían parte de la piel y el albedo. Las muestras de membrillo también mostraron una buena capacidad secuestrante de radicales. Fattouch y col., (2008) determinaron que la pulpa de membrillo presentó mayor efecto sobre el radical DPPH que la pulpa de pera y de manzana.

El poder reductor es un mecanismo de actividad antioxidante en el que se mide la conversión de un complejo ferrocianuro-Fe^{3+} a su forma ferrosa. El análisis de las propiedades reductoras mostró que el zumo de granada a una concentración de 7.5 g/100 g presentó la mayor AA. Entre las pastas y aguas de escaldado con mayor actividad, QBW y CBW mostraron similares capacidades de reducir hierro, no encontrándose diferencias significativas entre ellas, y además de un modo concentración-dependiente. Mediante este mecanismo las distintas pastas apenas presentaron AA.

Tabla 19. Actividad antioxidante de las distintas materias primas a diferentes concentraciones (A = 2.5%, B = 5%, C = 7.5%, D = 10%).

MATERIA PRIMA	**DPPH· Inhibición (%)**					**FRAP TEAC[1] (mM Trolox/L)**				**FIC Efecto quelante (%)**				
	A	B	C	D	$^{2}IC_{50}$	A	B	C	D	A	B	C	D	$^{3}EC_{50}$
Pasta														
Membrillo	4.22^{a}	6.81^{a,bB}	8.72^{bC}	12.69^{cB}	19.61	0.07^{aC}	0.10^{bC}	0.15^{cB}	0.19^{dB}	S.e	S.e	S.e	S.e	---
	(0.61)*	(0.61)	(0.08)	(1.00)		(0.00)	(0.00)	(0.00)	(0.01)					
Medjoul	1.93	1.25^{A}	2.24^{A}	3.99^{A}	34.71	0.02^{aA}	0.04^{a,bA}	0.05^{b,cA}	0.07^{cA}	S.e	S.e	S.e	S.e	---
	(0.15)	(0.69)	(0.46)	(0.99)		(0.00)	(0.00)	(0.00)	(0.00)					
Confitera	3.69	3.69^{A,B}	4.22^{B}	4.91^{A}	69.28	0.04^{aB}	0.05^{a,bB}	0.06^{bA}	0.08^{cA}	S.e	S.e	S.e	S.e	---
	(0.39)	(0.08)	(0.31)	(1.15)		(0.00)	(0.00)	(0.00)	(0.00)					
Agua de escaldado														
Membrillo	9.74^{aB}	14.24^{aB}	21.32^{bB}	22.70^{bC}	24.21	0.24^{a}	0.46^{bB}	0.57^{bB}	0.93^{cB}	S.e	S.e	S.e	S.e	---
	(0.23)	(0.14)	(2.07)	(0.05)		(0.06)	(0.03)	(0.02)	(0.04)					
Medjoul	5.60^{A}	6.71^{A}	6.66^{A}	7.77^{A}	41.51	0.09	0.09^{A}	0.14^{A}	0.22^{A}	1.45^{aA}	3.64^{bA}	4.45^{cA}	9.73^{dA}	17.07
	(0.47)	(0.53)	(0.16)	(0.22)		(0.02)	(0.00)	(0.00)	(0.05)	(0.03)	(0.02)	(0.04)	(0.04)	
Confitera	9.60^{aB}	15.16^{bB}	17.37^{cB}	17.18^{cB}	61.75	0.28^{a}	0.47^{bB}	0.66^{cB}	0.93^{dB}	17.82^{aB}	26.73^{bB}	32.82^{cB}	47.18^{dB}	10.52
	(0.28)	(0.51)	(0.14)	(0.23)		(0.01)	(0.03)	(0.02)	(0.02)	(0.10)	(0.04)	(0.04)	(0.05)	
Zumo de granada	23.52^{a}	38.31^{b}	47.27^{c}	72.76^{d}	7.47	0.57^{a}	1.18^{b}	1.50^{c}	0.74^{a}	3.82^{a}	7.09^{b}	18.64^{c}	22.45^{d}	12.53
	(0.23)	(1.06)	(0.28)	(0.32)		(0.00)	(0.10)	(0.01)	(0.00)	(0.01)	(0.02)	(0.10)	(0.02)	

[1]TEAC, capacidad antioxidante en equivalentes de Trolox; $^{2}IC_{50}$, concentración (%) para un 50% de inhibición; $^{3}EC_{50}$, concentración (%) para un 50% de efecto quelante. Valores en la misma fila con distinta letra pequeña difieren significativamente ($p<0.05$). Valores en la misma columna con distinta letra grande difieren significativamente ($p<0.05$).

*Valores en paréntesis indican error estándar. S.e., Sin efecto.

La habilidad quelante es de gran importancia en la industria alimentaria ya que los metales de transición contribuyen a la oxidación lipídica, la cual es la principal causa de degradación de alimentos (Viuda-Martos y col., 2010b). Por lo que es de gran importancia averiguar qué materias primas tienen la capacidad de quelar hierro (II). En el presente estudio ninguna de las pastas analizadas ni el agua de escaldado de membrillo presentó dicha habilidad. A mayor efecto quelante menor EC_{50}; en este sentido el orden de AA sería: CBW>PGJ>MBW.

Teniendo en cuenta los resultados obtenidos de los distintos métodos usados el zumo de granada fue la materia prima que mostró una mayor AA, además del elevado contenido en compuestos fenólicos, siendo en este caso la más apropiada para evaluar su efecto antioxidante en yogur enriquecido.

4.2.2. CARACTERIZACIÓN DE LOS YOGURES ENRIQUECIDOS

Los resultados de la caracterización del yogur enriquecido en agua de escaldado de dátil (Medjoul y Confitera) se presenta en el artículo publicado en la revista *Food and Bioproducts Processing* (2012, 90: 506-514); la caracterización del yogur enriquecido en agua de escaldado de membrillo se encuentra publicada en el *LWT - Food Science and Technology* (2011, 44: 1388-1395) y la caracterización del yogur enriquecido en zumo de granada se presenta en dos artículos, uno de ellos publicado en el *Journal of Agricultural and Food Chemistry* (DOI: 10.1021/jf501503h) y el otro publicado en la revista *Milchwissenschaft* (2012, 67(2): 177-180).

4.2.2.1. Curva de acidificación

La **Figura 6** muestra la evolución del pH durante la fermentación de los distintos yogures enriquecidos. El enriquecimiento de los yogures con las aguas de escaldado de membrillo y dátil Confitera estimula el proceso de fermentación, mientras que el zumo de granada y el agua de escaldado de dátil Medjoul aumentan el pH del producto final respecto a los yogures control ($p<0.05$). Cabe destacar que el yogur que contiene CBW acelera el proceso de fermentación en 30 minutos con respecto al resto de yogures ($p<0.05$), lo cual se confirma con el elevado contenido en ácido láctico en este yogur a

día 1 de almacenamiento en refrigeración (1396.04 mg/100 g). Esto puede ser debido a que CBW proporciona ácido fórmico (52.40 mg/100 g) el cual puede estimular el crecimiento de lactobacilos (Bautista y col., 1996).

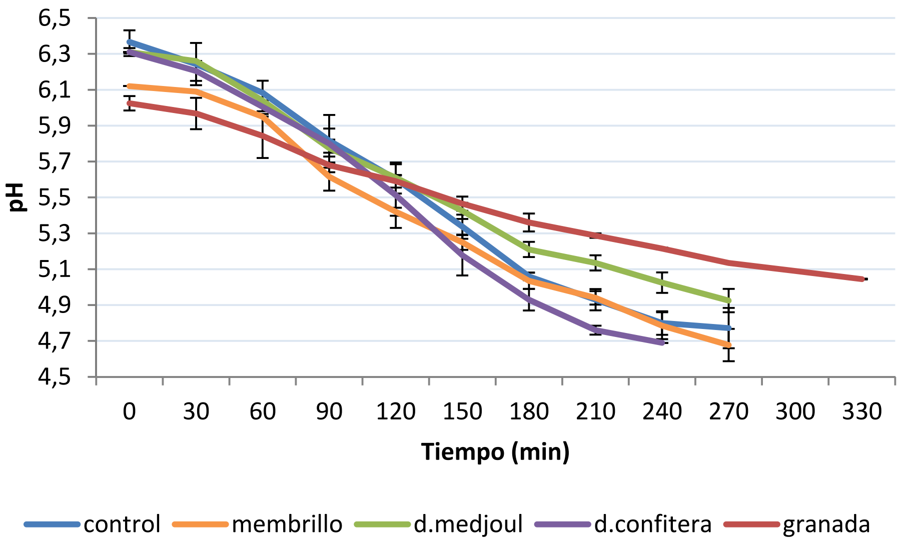


Figura 6. Curva de acidificación de la elaboración de los yogures.

La adición de zumo de granada retrasó el desarrollo del ácido con la consiguiente disminución de la bajada de pH. La repentina bajada de pH inicial (0.32 puntos) así como el tratamiento térmico de la leche reconstituida al 25% en sólidos pudo haber causado un efecto de pre-acidificación en la mezcla. En esta situación Phadungath (2005) estableció mayores valores finales de pH debido a la desnaturalización de las proteínas séricas, con lo que el punto isoeléctrico es mayor resultando en un proceso de solidificación a un pH mayor al esperado. Öztürk y Öner, (1999) observaron un comportamiento similar cuando adicionaban zumo de uva concentrado a yogur. Esto puede dar lugar a problemas de sinéresis y a la ruptura de las relaciones simbióticas entre los cultivos iniciadores.

4.2.2.2. Color

El color es la primera característica sensorial percibida por los consumidores y tiende a modificar otras percepciones tales como flavor y aroma (García-Pérez y col., 2005). La adición de las aguas de escaldado no produjo efectos significativos sobre los valores de *L** y *b**, únicamente la coordenada *a** se ve afectada por la adición de agua de

escaldado de dátil Confitera ($p<0.05$) (**Tabla 20**). Todas las muestras obtuvieron mayores valores de las coordenadas a* (menos verde) y b* (más amarillo) comparadas con las muestras control, lo cual puede ser debido a que todas las aguas de escaldado presentaban un tono marrón, pero no lo suficientemente elevado como para producir grandes cambios en las propiedades del color del producto final. La adición de fibra de pasta de dátil a yogur sí obtuvo un efecto significativo sobre los parámetros del color en el estudio llevado a cabo por Hashim y col., (2009).

La adición de zumo de granada afectó a todas las coordenadas de color (disminuyó *L**, aumentó *a** y disminuyó *b**). La adición de PGJ proporciona al yogur un color rojizo el cual es percibido como más atractivo por los consumidores. Gambaro y col., (2001) mostraron una influencia positiva en la aceptabilidad de yogur de fresa debido a la intensidad del color rojo.

Tabla 20. Color y textura de los yogures enriquecidos (valor medio ± error estándar).

Tipo de yogur	*L**	*a**	*b**	F_{max} (N)
CONTROL	$84,27 \pm 1,11^{b}$	$-2,74 \pm 0,06^{a}$	$5,34 \pm 0,16^{b}$	$32,56 \pm 1,04^{b}$
MEMBRILLO	$83,07 \pm 2,48^{b}$	$-2,21 \pm 0,23^{a,b}$	$6,15 \pm 0,49^{b}$	$20,52 \pm 0,71^{a}$
D. MEDJOUL	$85,46 \pm 1,91^{b}$	$-2,42 \pm 0,07^{a,b}$	$5,56 \pm 0,17^{b}$	$32,60 \pm 0,57^{b}$
D. CONFITERA	$82,71 \pm 0,78^{b}$	$-1,59 \pm 0,18^{b}$	$6,66 \pm 0,24^{b}$	$23,90 \pm 1,16^{a}$
GRANADA	72.19 ± 1.06^{a}	8.05 ± 0.29^{c}	1.77 ± 0.06^{a}	33.22 ± 2.23^{b}

Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$)

4.2.2.3. Textura

Los yogures enriquecidos en aguas de escaldado de membrillo y Confitera presentaron significativamente menores valores de F_{max} que los yogures control, Medjoul y granada, lo que indica que la estructura de los yogures control, Medjoul y granada es más dura que la de los yogures de membrillo y Confitera (**Tabla 20**). Las propiedades texturales de los geles ácidos están influenciadas por varios factores tales como: concentración de caseínas, pH, temperatura, la evolución del pH y temperatura y los sólidos totales (Phadungath, 2005). Con lo que el incremento en sólidos totales por parte de MBW (6.14%) y PGJ (14.42%) pudo haber aumentado la firmeza de los yogures.

En el yogur la fuerza de la red proteica aumenta conforme aumenta la liberación de ácido láctico y exopolisacáridos producidos por las bacterias lácticas (Lubbers y col., 2004). Por lo que el reblandecimiento de los yogures enriquecidos en QBW pudo ser debido a las bajas poblaciones de lactobacilos (**Figura 9**). Además el bajo pH de QBW (4.83) y CBW (5.70) causó una bajada repentina en el pH inicial de las mezclas que junto con el tratamiento térmico empleado pudo haber causado un efecto de pre-acidificación. En estas condiciones Peng y col., (2009) mostraron un aumento en la solubilización del fosfato cálcico coloidal, un aumento en la pérdida temprana del entrecruzamiento del fosfato cálcico coloidal lo que lleva a la producción de geles más débiles.

4.2.2.4. Reología

Los ensayos oscilatorios permiten evaluar las características de los yogures en condiciones no destructivas de la estructura. A partir de los resultados de las pruebas oscilatorias se calcularon los módulos de elasticidad y pérdida, G' y G'', los cuales están relacionados con la energía almacenada y liberada, respectivamente, por ciclo de deformación, mientras que la tan (δ) está asociada con el grado de viscoelasticidad de la muestra (Singh y Muthukumarappan, 2008). Los yogures firmes poseen propiedades de flujo que son características de fluidos viscoeláticos débiles y no-Newtonianos con un comportamiento altamente tiempo-dependiente (Ares y col., 2006). Como el yogur es un producto vivo, los cambios estructurales pueden suceder en diversas etapas entre la incubación y el almacenamiento en frío. De hecho, la fuerza de la red proteica aumenta conforme aumenta el contenido en ácido láctico y la producción de exopolisacáridos producidos por las bacterias vivas del yogur (Lubbers y col., 2004).

Los yogures estudiados permanecen en la región viscoelástica lineal durante todo el rango de frecuencias ensayadas (0.01-1 Hz). Se seleccionarion los siguientes parámetros para llevar a cabo el análisis estadístico: G', G'', η^*, tan (δ) y γ (**Tabla 21**).

Coincidiendo con los resultados obtenidos por Sendra y col., (2010) los yogures muestran un comportamiento predominantemente elástico ($G' > G''$) durante todo el rango de frecuencias ensayadas, lo que se corresponde próximamente al comportamiento de un gel verdadero. En general, los módulos (G' y G'') aumentan

Tabla 21. Evolución de los parámetros reológicos de los yogures enriquecidos en agua de escaldado de dátil y membrillo bajo prueba oscilatoria (valores del barrido de frecuencia obtenidos a 0.1 Hz, 0.4 Pa, 7°C): *G'*, módulo de elasticidad; *G''*, módulo de viscosidad; *η**, viscosidad compleja; tan (*δ*) *y γ* deformación durante 28 días de almacenamiento en refrigeración.

Tipo de yogur	Día	***G'* (Pa)**		***G''* (Pa)**		***η** (Pa·s)**		**tan *δ* (-)**		***γ* (-)**	
		Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.
Control	1	721.05	39.75	162.70	7.20	1175.00	65.00	0.226	0.003	0.00054	0.00003
	7	753.70	153.71	163.20	35.21	1229.00	250.99	0.230	0.010	0.00057	0.00008
	14	1264.47	309.76	294.90	84.55	2066.67	511.25	0.215	0.005	0.00034	0.00013
	21	1160.33	216.71	245.27	44.49	1886.67	352.44	0.212	0.001	0.00036	0.00006
	28	1179.00	87.88	247.93	17.29	1916.67	141.11	0.210	0.003	0.00034	0.00026
Membrillo	1	417.80	43.18	102.70	5.28	685.00	63.24	0.246	0.002	0.00093[b]	0.00003
	7	705.73	69.09	173.17	18.74	1156.67	112.60	0.245	0.005	0.00056[a]	0.00005
	14	790.00	13.60	188.40	4.40	1295.00	25.00	0.239	0.002	0.00049[a]	0.00001
	21	789.97	116.36	183.50	26.93	1293.00	191.29	0.232	0.002	0.00052[a]	0.00008
	28	640.55	28.95	150.80	4.10	1045.00	45.00	0.236	0.005	0.00061[a]	0.00003
Medjoul	1	444.00[a]	11.02	104.10[a]	4.35	726.00[a]	12.07	0.234[b]	0.002	0.00088[d]	0.00001
	7	1045.00[d]	14.00	244.25[d]	0.55	1710.00[d]	20.00	0.234[b]	0.003	0.00037[a]	0.00001
	14	986.85[c,d]	67.15	222.35[c,d]	13.95	1610.00[c,d]	110.00	0.226[a,b]	0.002	0.00040[a,b]	0.00003
	21	854.80[b,c]	23.70	185.15[b,c]	7.95	1390.00[b,c]	40.00	0.217[a]	0.004	0.00046[b,c]	0.00001
	28	785.20[b]	9.60	169.50[b]	3.40	1275.00[b]	15.00	0.216[a]	0.002	0.00050[c]	0.00001
Confitera	1	1286.00	42.00	312.15	12.05	2110.00	70.00	0.243[b]	0.002	0.00030	0.00001
	7	1598.00	178.97	371.73	35.46	2610.00	287.89	0.234[a,b]	0.007	0.00025	0.00003
	14	1283.78	166.10	284.92	39.74	2090.00	271.85	0.221[a,b]	0.003	0.00033	0.00005
	21	1053.85	109.84	234.35	27.99	1720.00	181.52	0.221[a,b]	0.005	0.00038	0.00005
	28	1107.13	109.54	233.28	22.31	1802.50	176.42	0.211[a]	0.006	0.00036	0.00004

E. E., error estándar.
Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

conforme aumenta la frecuencia mostrando características de una solución concentrada (Staffolo y col., 2004). Nuestros valores experimentales para tan (δ) varían entre 0.210 a 0.246 lo cual señala a un polímero concentrado amorfo (Steffe, 1996); un valor bajo de tan (δ) indica que el gel tiene un predominante carácter elástico (Singh y Muthukumarappan, 2008).

El tipo de yogur afectó significativamente ($p<0.05$) a G', η^*, tan (δ) y γ mientras que el tiempo de almacenamiento sólo afectó ($p<0.05$) a la tan (δ) y a γ. El almacenamiento de las muestras durante dos semanas no causó ningún cambio significativo en las propiedades viscoelásticas de las muestras con respecto al primer día. Sin embargo, a día 21 y 28, la tan (δ) fue estadísticamente menor a los días 1 y 7. Esto indica un incremento en las características sólidas de las muestras tras dos semanas de almacenamiento, lo cual puede ser debido al incremento de la acidez de las muestras durante dicho periodo. En cuanto al tipo, los yogures control y Confitera mostraron mayores valores de G' y G'' y fueron los que presentaron menor pH, especialmente los yogures enriquecidos en agua de escaldado Confitera los cuales además presentaban la mayor viscosidad. A su vez presentan mayores recuentos de estreptococos (**Figura 9**) capaces de liberar exopolisacáridos que contribuyen al reforzamiento de la estructura del gel. Respecto a los yogures con zumo de granada, no se observaron diferencias significativas con el control en ninguno de los parámetros reológicos determinados. De este modo podemos concluir que las pequeñas diferencias observadas en los parámetros reológicos se debieron a las diferencias en el pH entre los yogures.

4.2.2.5. Análisis sensorial

Las pruebas afectivas (como los tests de aceptabilidad) nos proporcionan valiosa información sobre el potencial comercial el producto desarrollado ya que la opinión de los consumidores es de gran importancia en el desarrollo de un nuevo producto alimentario y conocer los gustos de los consumidores es la clave para colocar un producto en el mercado (Cardarelli y col., 2008). Existen diferencias significativas en la apariencia, flavor y aceptación general entre los yogures control y los enriquecidos. La cremosidad, dulzor, astringencia y acidez fueron puntuados de manera muy similar en todas las muestras. Los yogures enriquecidos en PGJ y QBW mayores puntuaciones en

el atributo apariencia mientras que el enriquecimiento con PGJ y MBW mejoraron la percepción del flavor ($p<0.05$). Los yogures control y granada presentaron los mayores valores de aceptación general mientras que los yogures enriquecidos en QBW fueron los peor valorados con respecto a este atributo. El membrillo es un fruto que aun estando en su estado óptimo de maduración se caracteriza por su elevado amargor y astringencia, principalmente dadas por su contenido en compuestos fenólicos (Wojdyło y col., 2013), lo que hace que sea un producto de escaso consumo en fresco. Entre las aguas de escaldado, CBW y QBW presentaron el mayor contenido en fenoles y flavonoides totales (*Véase* sección **5.2.1.4. Contenido en fenoles y flavonoides totales**); este hecho junto con su bajo contenido en azúcares totales (1.20% y 2.70%, respectivamente) se ha reflejado en las puntuaciones obtenidas en el análisis sensorial. Viuda-Martos y col., (2009, 2010c) incorporaron satisfactoriamente las aguas de lavado de la industria de cítricos a un producto cárnico con buenos resultados sensoriales.

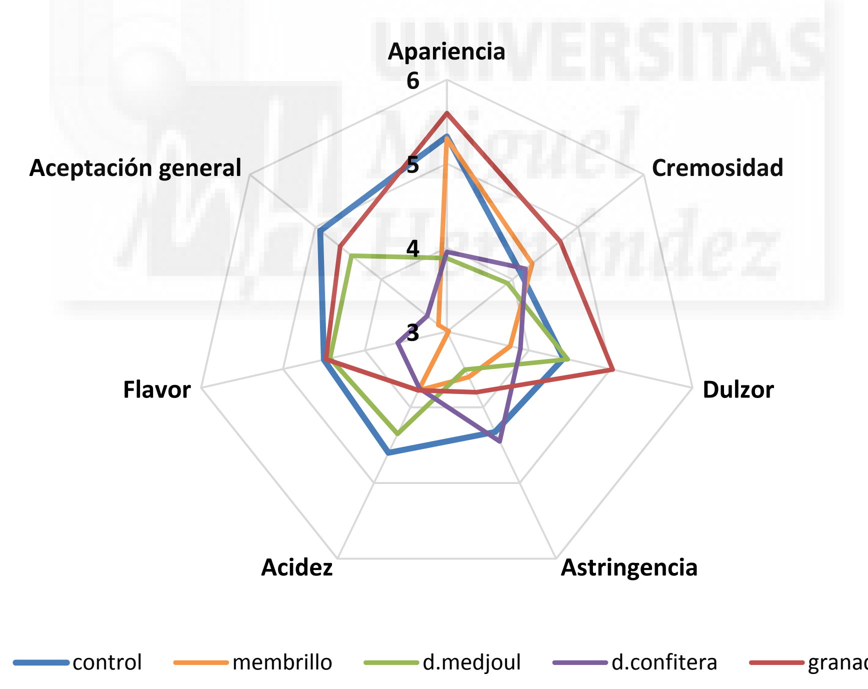


Figura 7. Evaluación sensorial de los yogures enriquecidos (escala hedónica de 7 puntos).

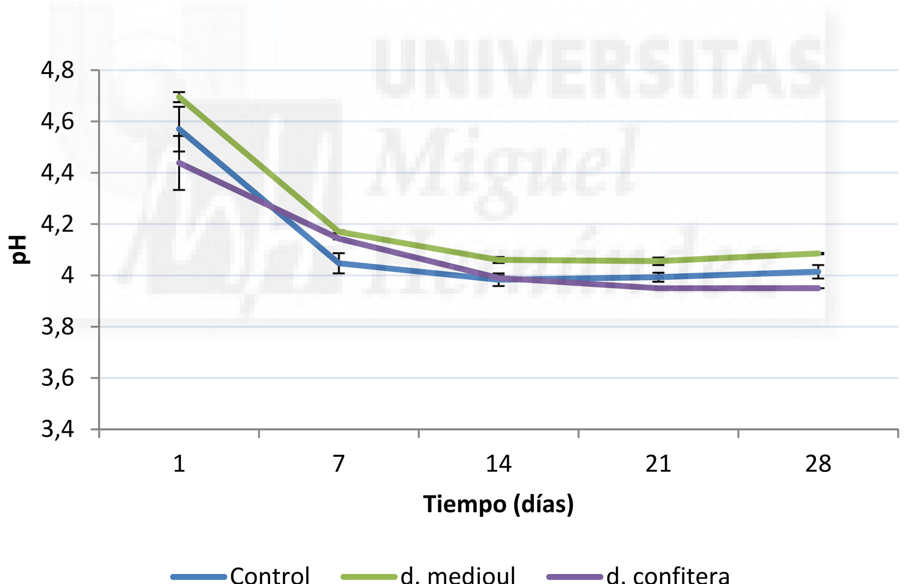
La satisfacción de consumidor ante un yogur está directamente correlacionada con atributos sensoriales específicos tales como dulzor, textura y color en yogures de frutas y acidez en yogures sin sabores (Gambaro y col., 2001). En general entre los yogures enriquecidos el yogur con zumo de granada fue el más aceptado por parte de

los consumidores. En este tipo de yogur la adición del 8% de azúcar no hubiera sido necesaria pues los azúcares propios del zumo aportaban la cantidad adecuada para el agrado de los consumidores.

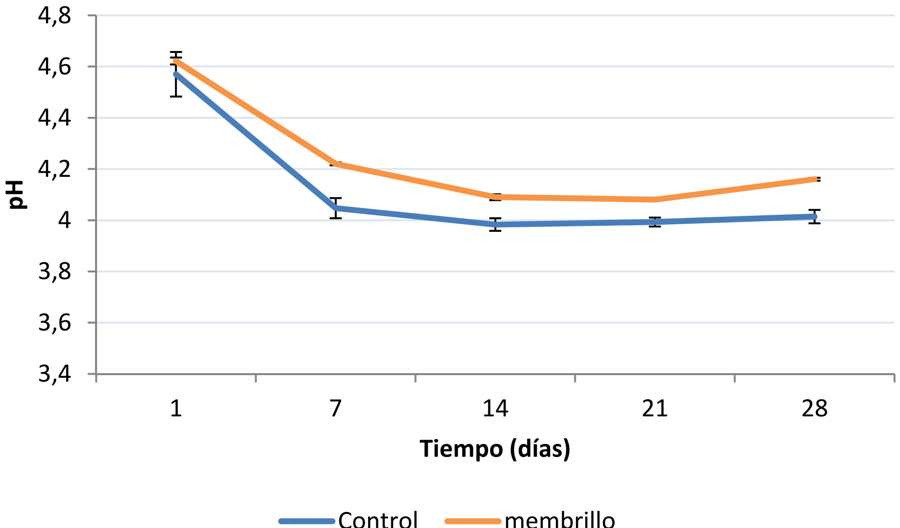
4.2.2.6. Evolución del pH y recuentos microbiológicos de los yogures durante el almacenamiento en refrigeración

Los yogures enriquecidos en MBW, QBW y PGJ presentaron mayores valores de pH ($p<0.05$) que los yogures control y CBW. A lo largo del almacenamiento a 4°C se observaron diferencias significativas en los valores de pH. El pH disminuyó gradualmente (**Figura 8**) en todos los yogures durante el periodo de almacenamiento, debido presumiblemente a la continuada fermentación de las bacterias lácticas (Dave y Shah, 1997).

(a)



(b)



(c)

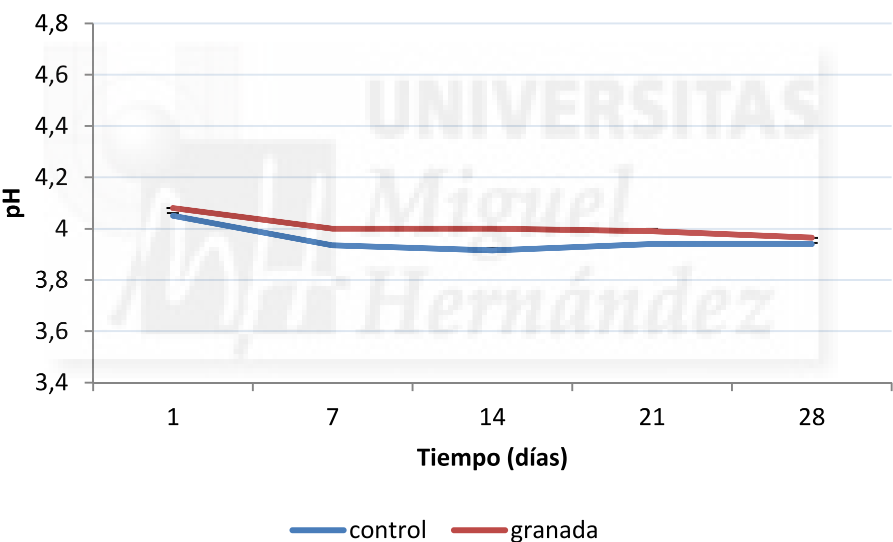


Figura 8. Evolución del pH de los yogures enriquecidos en (a) aguas de escaldado de dátil Medjoul y Confitera, (b) en agua de escaldado de membrillo y (c) en zumo de granada.

A lo largo del almacenamiento los recuentos de estreptococos fueron superiores a los de lactobacilos en todos los casos (**Figura 9**). En los yogures enriquecidos en aguas de escaldado de dátil (**Figura 9a**), los recuentos de estreptococos en los yogures enriquecidos en CBW fueron significativamente mayores que en los enriquecidos en MBW; además, los mayores recuentos de lactobacilos ($p<0.05$) se dieron en los

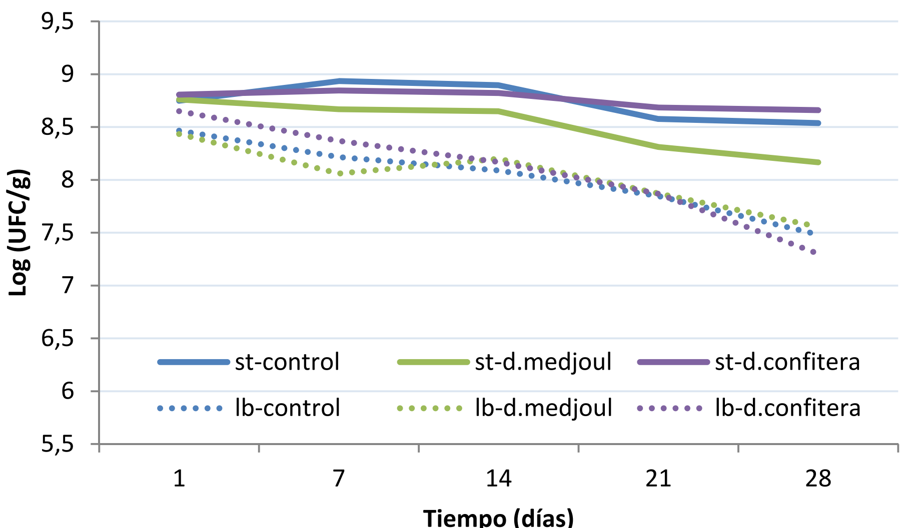
yogures confitera. Esto está correlacionado con el bajo pH de los yogures confitera y explicado por la presencia natural de ácido fórmico en la materia prima inicial.

En cuanto a los yogures enriquecidos en QBW tanto los recuentos de estreptococos como los de lactobacilos fueron superiores en los yogures control ($p<0.05$) (**Figura 9b**), lo cual está correlacionado con el bajo pH de los estos yogures. Estudios previos han explicado que los principales factores para la pérdida de la viabilidad celular son el descenso del pH durante el almacenamiento del producto (post-acidificación) y la acumulación de ácidos orgánicos como resultado del crecimiento y fermentación (Kailasapathy y col., 2008). Aunque el pH no es en este caso un factor limitante debido a la capacidad tamponante de la leche, el QBW es rica en ácidos orgánicos especialmente en acético y fórmico que en este caso podrían haber contribuido al efecto inhibitorio de QBW sobre los lactobacilos. De hecho, Fattouch y col., (2008) mostraron el efecto antibacteriano del membrillo (extracto de la piel) sobre *Staphylococcus aureus*, *Pseudomonas aeruginosa* y *Bacillus cereus* a una concentración inhibitoria relativamente baja (de 10^2 a 10^4 µg polifenoles/mL); y específicamente en el estudio de Karar y col., (2013) el ácido quínico y un derivativo del ácido quínico provenientes de un extracto en crudo de membrillo mostraron una fuerte actividad inhibitoria sobre *Escherichia coli*.

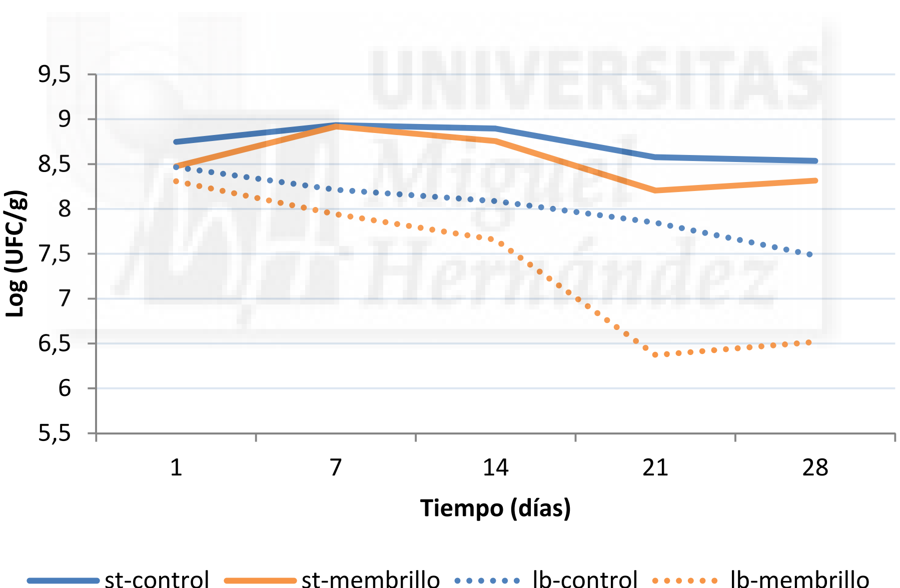
En el caso de los yogures con zumo de granada, al igual que en el caso anterior, los recuentos de estreptococos y lactobacilos fueron superiores en los yogures control ($p<0.05$), e igualmente correlacionado con el bajo pH de estos yogures. Está demostrado que un exceso de sólidos solubles (en forma de zumo concentrado) inhibe el crecimiento de las bacterias lácticas (Celik y Bakirci, 2003; Öztürk y Öner, 1999). Además Ramaswamy y Basak (1992) mostraron que elevadas cantidades de glucosa en el medio y elevada presión osmótica pueden causar pérdida de viabilidad.

Tanto en los yogures con las aguas de escaldado de dátil como en los yogures con zumo de granada los recuentos de bacterias lácticas excedieron los requerimientos mínimos de células viables en el momento de consumo que establece la legislación española (10^7) durante todo el tiempo de almacenamiento.

(a)



(b)



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(c)

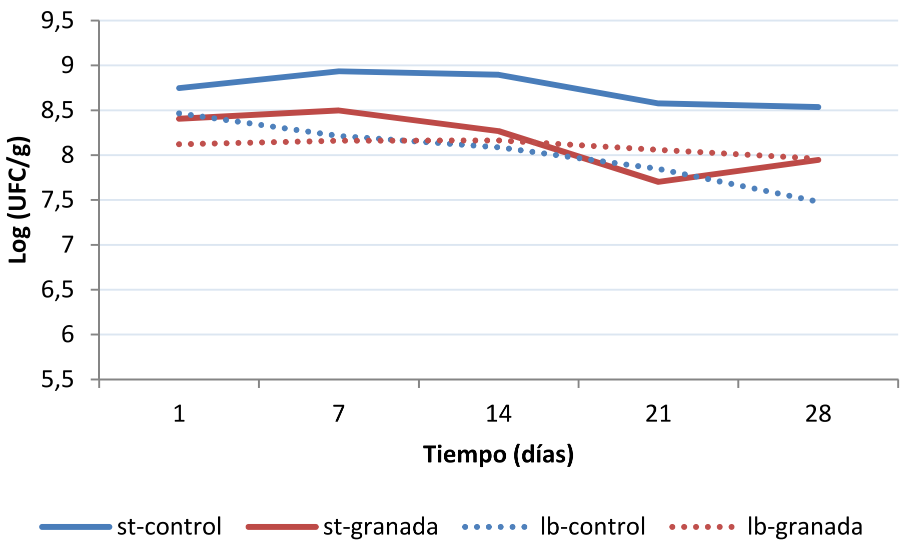

Figura 9. Evolución del recuento de estreptococos (st) y lactobacilos (lb) de los yogures enriquecidos en (a) aguas de escaldado de dátil Medjoul y Confitera, (b) agua de escaldado de membrillo y (c) zumo de granada.

4.2.2.7. Evolución del contenido en ácidos orgánicos y azúcares de los yogures durante el almacenamiento en refrigeración

La **Tablas 22** y **23** muestran los cambios en el contenido en ácidos orgánicos y azúcares de los yogures enriquecidos en aguas de escaldado de dátil y membrillo durante su almacenamiento en refrigeración. Los ácidos orgánicos son indicadores importantes de la actividad metabólica de las bacterias lácticas y contribuyen al sabor y flavor del yogur junto con otros compuestos volátiles y semi-volátiles tales como diacetilo y acetaldehído (Adhikari y col., 2002). Todos los ácidos orgánicos detectados se vieron significativamente afectados por el tipo de yogur. Los yogures enriquecidos en MBW presentaron mayor concentración de ácido oxálico ($p<0.05$) que los enriquecidos en QBW y CBW (sin diferencias significativas entre ellos) y los yogures control; aunque esta diferencia fue pequeña. La concentración en ácido cítrico fue algo menor ($p<0.05$) en los yogures control. El ácido cítrico está presente en la leche como producto del metabolismo bovino (Serra y col., 2009); además el ácido cítrico es consumido durante el proceso de fermentación, sin embargo apenas fue utilizado durante el almacenamiento. Como era de esperar el ácido láctico fue el ácido mayoritario,

encontrándose en mayor concentración en los yogures enriquecidos en CBW ($p<0.05$) lo cual está relacionado con los mayores recuentos de lactobacilos en los yogures confitera (**Figura 9**). El ácido acético se detectó en mayor concentración en los yogures enriquecidos en membrillo lo cual se mantiene por su elevado contenido en el agua de escaldado inicial. Además el ácido acético puede producirse a partir de citrato, lactosa y amino ácidos (Ong y Shah, 2009).

Únicamente en los yogures enriquecidos en QBW se detectó la presencia de ácidos málico, fumárico y ascórbico (*Véase* publicación "Production of low-fat yogurt with quince (*Cydonia oblonga* Mill.) scalding water") aunque estuvieron presentes en menores cantidades que en el agua de escaldado inicial.

No se detectaron diferencias significativas en el contenido en ácidos orgánicos de los yogures durante su almacenamiento excepto para el ácido láctico el cuál aumentó su concentración ($p<0.05$) los días 7 y 28 con respecto al día 1 de almacenamiento en refrigeración. La acidez total aumentó en todos los yogures a lo largo del almacenamiento, aunque no de forma significativa.

De nuevo se detectó un pico importante en el tiempo de retención del ácido tartárico, si fuese cuantificado su concentración variaría entre 221.82 a 320.00 mg/100 g.

Respecto a los azúcares, el contenido en lactosa disminuyó en los yogures durante el periodo de almacenamiento (**Tabla 23**). La galactosa aumentó o permaneció constante en todos los yogures ($p>0.05$) durante el almacenamiento en refrigeración. Esto se debe a que los cultivos utilizados en la fermentación del yogur (*L. bulgaricus* y *S. thermophilus*) utilizan la glucosa liberada de la lactosa pero no la galactosa. Por ello, mientras que el contenido en lactosa y glucosa disminuyen en el producto fermentado, la concentración de galactosa prácticamente no varía (O'Brien, 1999). El contenido en lactosa es mucho menor ($p<0.05$) en los yogures control que en el resto de yogures enriquecidos. Como las aguas de escaldado aportan glucosa a la leche desnatada (también sacarosa y fructosa), ésta es detectada en los yogures enriquecidos pero en menores cantidades que en las aguas de escaldado, siendo estadísticamente superior en los yogures enriquecidos en QBW. Entre los dátiles, los yogures enriquecidos en MBW contenían mayores cantidades de maltohexaosa, glucosa y galactosa que los

Tabla 22. Evolución de los ácidos oxálico, cítrico, láctico y acético (mg/100 g) de los yogures enriquecidos en aguas de escaldado de dátil y membrillo durante 28 días de almacenamiento en refrigeración.

Tipo de yogur	**Día**	**Oxálico**		**Cítrico**		**Láctico**		**Acético**		***Acidez total (%)***	
		Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.
Control	1	1.49^{b}	0.08	198.94	13.02	1192.56	55.72	11.13^{a}	0.39	1.67	0.14
	7	1.49^{b}	0.06	183.21	6.02	1333.30	54.73	12.75^{a}	0.60	1.76	0.02
	14	$1.23^{a,b}$	0.09	177.96	10.89	1294.17	107.65	$13.33^{a,b}$	0.88	1.71	0.21
	21	1.09^{a}	0.10	171.21	11.07	1306.54	98.79	$15.19^{b,c}$	0.80	1.73	0.19
	28	1.14^{a}	0.03	187.65	9.79	1383.85	54.59	16.01^{c}	1.12	1.84	0.17
Membrillo	1	3.54	0.11	257.82	1.08	1364.51	89.65	50.53	4.95	1.99	0.07
	7	3.11	0.70	274.69	46.80	1612.28	289.75	63.11	12.53	2.32	0.40
	14	1.62	0.53	180.73	7.38	1196.45	24.01	42.67	1.21	1.69	0.05
	21	1.57	0.71	182.52	21.81	1318.41	156.18	49.74	6.25	1.81	0.21
	28	2.82	0.56	249.17	32.79	1648.53	179.96	65.01	6.76	2.31	0.26
Medjoul	1	2.59	0.09	228.27	8.89	1247.74	15.23	20.95	11.37	1.76	0.02
	7	3.76	0.12	254.35	13.02	1592.51	78.97	12.68	1.81	2.14	0.10
	14	3.50	0.04	223.23	1.75	1482.32	40.54	12.98	1.63	1.99	0.06
	21	4.95	1.80	240.86	16.97	1581.82	128.32	15.82	1.81	2.16	0.13
	28	6.22	0.13	248.21	17.96	1567.96	56.88	16.05	0.66	2.18	0.13
Confitera	1	2.12	0.17	256.54	16.98	1396.04	114.00	10.66^{a}	1.07	2.04	0.16
	7	2.48	0.02	268.13	7.66	1629.81	23.68	$14.03^{a,b}$	1.41	2.26	0.02
	14	2.42	1.32	304.18	58.10	1570.58	101.60	$15.44^{a,b}$	1.66	2.21	0.15
	21	2.80	0.86	239.75	19.49	1580.29	89.39	17.20^{b}	0.39	2.16	0.11
	28	1.87	0.03	221.45	3.31	1509.94	51.35	$14.27^{a,b}$	0.61	2.06	0.06

E. E., error estándar.
Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

Tabla 23. Evolución de maltohexaosa y glucosa (mg/100 g), galactosa y lactosa (g/100 g) de los yogures enriquecidos en aguas de escaldado de dátil y membrillo durante 28 días de almacenamiento en refrigeración.

Tipo de yogur	**Días**	**Maltohexaosa**		**Glucosa**		**Galactosa+Fructosa**		**Lactosa**		***Azúcares totales (%)***	
		Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.
Control	1	84.66	3.43	N. d.	---	1.12	0.06	4.35	0.12	5.55	0.07
	7	76.61	1.82	N. d.	---	1.24	0.03	4.17	0.18	5.49	0.24
	14	72.67	7.12	N. d.	---	1.20	0.10	3.76	0.24	5.03	0.13
	21	72.21	7.26	N. d.	---	1.21	0.09	3.68	0.20	4.97	0.25
	28	83.07	2.48	N. d.	---	1.29	0.07	4.04	0.11	5.41	0.18
Membrillo	1	100.76	0.00	411.24	13.70	2.34	0.11	6.30	0.24	9.15	0.37
	7	102.77	19.24	416.94	84.25	2.58	0.52	6.29	1.42	9.38	2.05
	14	72.82	3.87	296.12	5.26	1.85	0.04	4.31	0.10	6.53	0.13
	21	72.36	12.96	291.93	29.64	1.95	0.22	4.27	0.50	6.58	0.77
	28	92.64	12.70	406.44	48.62	2.54	0.31	5.77	0.80	8.81	1.17
Medjoul	1	143.02	39.35	136.57	15.66	1.13	0.10	5.39	0.73	6.80	0.80
	7	124.05	2.70	161.46	8.88	1.54	0.77	6.34	0.34	8.17	0.43
	14	95.15	3.66	135.49	2.59	0.79	0.65	5.59	0.01	6.61	0.66
	21	95.62	15.69	157.27	14.98	1.51	0.15	5.63	0.70	7.38	0.88
	28	106.12	5.94	162.47	9.80	0.87	0.72	5.90	0.15	7.03	0.88
Confitera	1	90.76	2.43	40.15	3.00	1.26	0.09	5.70	0.35	7.46	0.08
	7	97.75	2.51	61.89	4.32	0.78	0.64	5.60	0.03	6.54	0.61
	14	108.24	21.57	83.08	12.82	1.46	0.15	5.27	0.34	6.92	0.52
	21	90.44	7.10	75.11	4.36	1.38	0.11	4.77	0.53	6.31	0.65
	28	88.09	2.57	78.46	0.15	1.30	0.04	4.47	0.13	5.93	0.17

E. E., error estándar; N. d., no detectado.

enriquecidos en CBW, lo cual está relacionado con el contenido en SST de las aguas de escaldado iniciales.

La evolución de los ácidos orgánicos y azúcares en los yogures enriquecidos en zumo de granada siguieron un patrón similar a los enriquecidos en las distintas aguas de escaldado. Entre los ácidos orgánicos, el ácido láctico fue el más abundante (**Tabla 24**) y su contenido fue aumentando durante el almacenamiento. La concentración de ácido acético disminuyó durante el almacenamiento ($p<0.05$), siendo el contenido significativamente mayor en los control. El ácido cítrico se mantuvo estable durante el almacenamiento. Los ácidos málico y fumárico únicamente se detectaron en los yogures con granada ya que el PGJ los aporta, aunque el ácido málico en mucha menor concentración en comparación a la del zumo inicial (748.80 mg/100 g).

La lactosa disminuyó en los yogures control pero apenas lo hizo en los yogures enriquecidos (**Tabla 25**). Según Tamime y Robinson (2007) cuando la leche es fortificada con un 14% en sólidos no grasos los yogures pueden contener entre 4-5 g lactosa/100 mL de yogur, como sucede en este estudio. El contenido en galactosa-fructosa (coeluyen en el cromatograma) fue mayor ($p<0.05$) en los yogures con granada, y su contenido aumentó en ambos yogures a lo largo del almacenamiento ya que no es metabolizada (Tamime y Deeth, 1980). El mayor contenido en galactosa de los yogures con granada es debido a que PGJ proporciona fructosa, y fructosa y galactosa co-eluyen en bajo las condiciones cromatográficas anteriormente descritas. Igualmente el PGJ aporta glucosa y por ello es detectada únicamente en los yogures enriquecidos.

Tabla 24. Evolución de los ácidos oxálico, cítrico, málico, láctico y fumárico (mg/100 g) de los yogures enriquecidos en zumo de granada durante 28 días de almacenamiento en refrigeración.

Tipo de yogur	**Día**	**Oxálico**		**Cítrico**		**Málico**		**Láctico**		**Fumárico**		***Acidez Total (%)***	
		Media	E.E.	Media	E. E.	Media	E. E.	Media	E. E.	Media	E.E.	Media	E.E.
Control	1	1.56[b]	0.04	208.18	4.11	N. d.	---	1293.93	35.57	N. d.	---	1,51	0.06
	7	1.41[a,b]	0.11	176.38	11.35	N. d.	---	1350.52	94.82	N. d.	---	1,54	0.12
	14	1.26[a,b]	0.04	193.81	0.43	N. d.	---	1453.01	62.22	N. d.	---	1,66	0.05
	21	1.18[a]	0.03	186.87	2.18	N. d.	---	1444.67	47.59	N. d.	---	1,65	0.03
	28	1.19[a]	0.00	200.26	11.76	N. d.	---	1514.69	40.66	N. d.	---	1,73	0.09
Granada	1	1.12[b]	0.07	209.40	17.04	83.19	10.36	1252.94	236.65	4.36	1.11	1,62	0.27
	7	0.26[a]	0.08	201.24	34.89	78.16	11.49	1190.11	213.77	1.85	0.51	1,49	0.30
	14	0.23[a]	0.04	217.36	3.67	89.28	2.25	1389.35	15.09	1.67	0.01	1,72	0.03
	21	0.18[a]	0.02	216.37	8.53	89.56	6.08	1436.77	61.47	1.62	0.11	1,77	0.08
	28	0.21[a]	0.03	212.72	9.62	83.85	4.38	1414.77	44.87	2.07	0.13	1,73	0.09

E. E., error estándar; N. d., no detectado.
Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

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Tabla 25. Evolución de maltohexaosa y glucosa (mg/100 g), galactosa y lactosa (g/100 g) de los yogures enriquecidos en zumo de granada durante 28 días de almacenamiento en refrigeración.

Tipo de yogur	**Día**	**Maltohexaosa**		**Glucosa**		**Galactosa + Fructosa**		**Lactosa**		***Azúcares Totales (%)***	
		Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.	Media	E. E.
Control	1	79.75	0.31	N.d.	---	1,20	0,03	4,33	0,07	5,61	0,10
	7	79.64	1.88	N.d.	---	1,26	0,08	3,91	0,26	5,25	0,34
	14	71.66	4.66	N.d.	---	1,33	0,06	3,76	0,17	5,15	0,23
	21	77.66	0.62	N.d.	---	1,33	0,04	3,81	0,11	5,21	0,15
	28	84.98	4.45	N.d.	---	1,40	0,03	4,09	0,13	5,58	0,16
Granada	1	77.90	7.30	1,78	0,16	3,05	0,27	4,70	0,41	9,61	0,84
	7	74.44	14.68	1,74	0,30	3,05	0,53	4,47	0,79	9,33	1,64
	14	80.23	8.16	1,89	0,03	3,40	0,05	4,80	0,08	10,17	0,17
	21	78.95	0.26	1,91	0,08	3,44	0,15	4,71	0,21	10,14	0,44
	28	86.68	7.69	1,89	0,06	3,40	0,11	4,63	0,16	10,01	0,34

N.d., no detectado; E. E., error estándar.

4.2.2.8. Perfil de compuestos fenólicos individuales en los yogures enriquecidos en zumo de granada y grado de interacción proteína-polifenoles

El zumo de granada fue el extracto que presentó la mayor actividad antioxidante *in vitro*; además su contenido en compuestos fenólicos fue considerable. Todo ello junto con el hecho de que fue el mejor valorado por parte de los consumidores hizo que el estudio de la partición de los compuestos fenólicos en el yogur así como su capacidad antioxidante se centrara únicamente en este tipo de yogur enriquecido.

La caracterización de los compuestos fenólicos mayoritarios del zumo de granada, las **antocianinas**, se presenta en la **Tabla 26** y en la **Figura 10** se muestran perfiles típicos de antocianinas de zumo de granada, yogur con zumo de granada y permeato de yogur.

Tabla 26. LC-QTOF/MS análisis de las antocianinas en zumo y yogur de granada.

Pico	Compuesto	R_t (min)	λ_{max} (nm)	$[M+H]^+$ (*m/z*)	MS/MS (*m/z*)
1	Delfinidin-3,5-*O*-diglucosido	5.70	277; 519	627	465/303/186
2	Cianidin-3,5-*O*-diglucosido	6.82	277; 513	611	449/287/186
3	Delfinidin-3-*O*-glucosido	7.25	277; 522	465	303/186
4	Pelargonidin-3,5-*O*-diglucosido	7.99	274; 499	595	433/274/303/186
5	Cianidin-3-*O*-glucosido	8.49	280; 516	449	287
6	Pelargonidin-3-*O*-glucosido	9.77	274; 503	433	271
7	Cianidin-pentosido	10.80	277; 513	419	287

Abreviaciones: R_t, tiempo de retención; λ_{max}, espectro máximo en UV-visible; MS, espectro de masa.

Se detectaron un total de 7 antocianinas, 3 cianidinas, 2 delfinidinas y 2 pelargonidinas, cada una de ellas asociadas por su forma monoglucosido o diglucosilada. Al igual que en el presente estudio, otros autores encontraron a las antocianinas como los compuestos mayoritarios en zumos de granada procedentes sólo de los arilos (Fischer y col., 2011; Gil y col., 2000; Qu y col., 2012). Otros compuestos como el ácido elágico y las punicalaginas fueron detectadas. El contenido en ácido elágico en PGJ fue de 21.17 mg/L, en yogur con granada a día 1 de almacenamiento fue de 8.58 mg/L y a día 28 fue de 11.75 mg/L. La importancia del ácido elágico es debida a que junto con con otros compuestos fenólicos como las

punicalaginas, punicalinas y ácido galágico es responsable de la actividad antioxidante *in vitro* del fruto de la granada (Johanningsmeier y Harris, 2011); además el ácido elágico posee propiedades antioxidantes (Falsaperla y col., 2005) y anticarcinogénicas (Hassoun y col., 2004) *in vivo*.

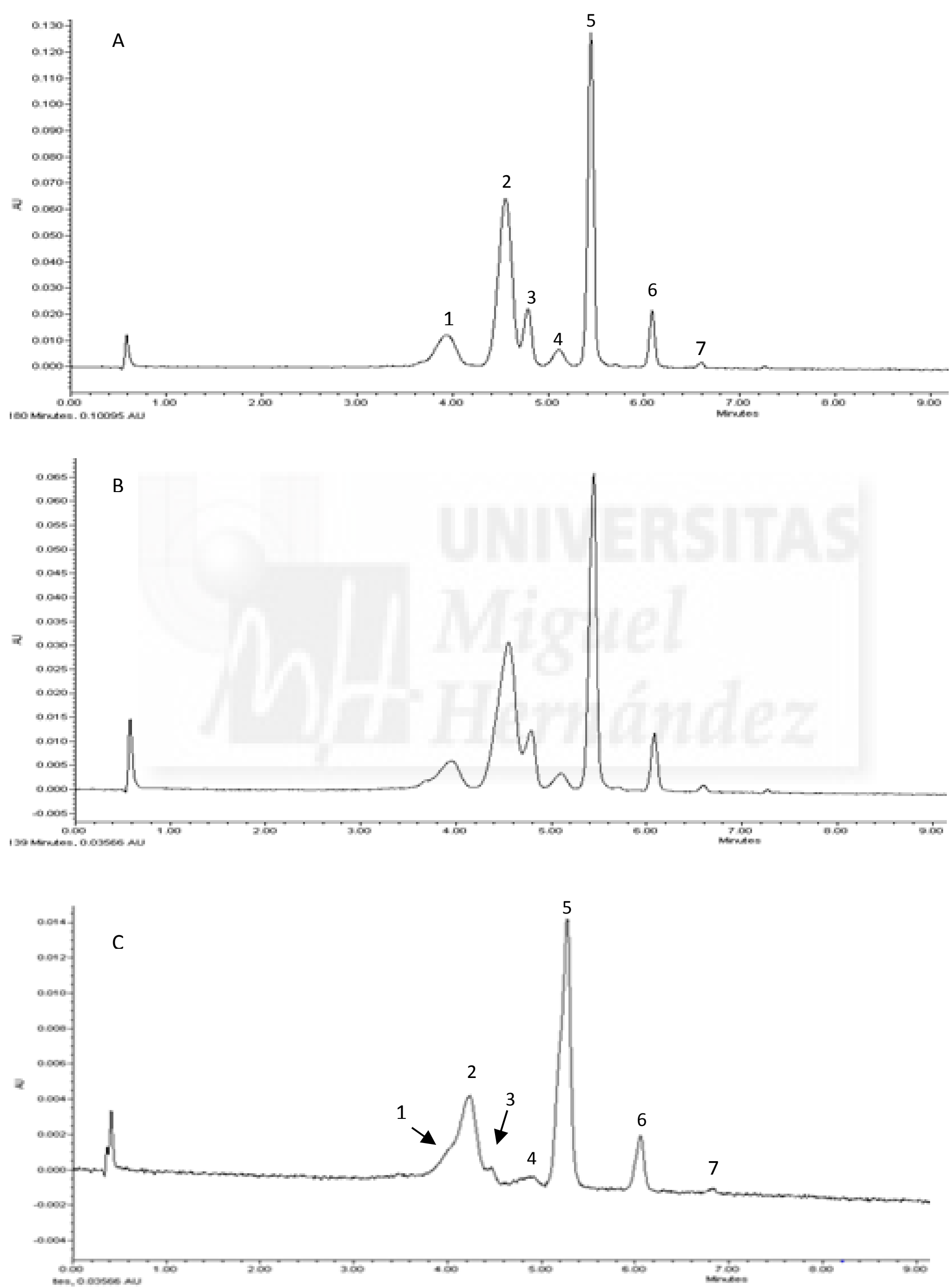

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Figura 10. Cromatogramas del perfil de antocianos en A) zumo de granada, B) yogur con zumo de granada y C) permeato de yogur con granada a 520 nm.

Con respecto al contenido en punicalaginas (α+β) en PGJ fue de 46.36 mg/L y en yogur con granada a día 1 y 28 de almacenamiento fue de 31.90 mg/L y 31.37 mg/L, respectivamente. El contenido siempre fue superior en el anómero β para todas las muestras, lo cual es característico de zumos no comerciales (Mena y col., 2011). Muchos autores atribuyen la elevada actividad antioxidante del PGJ a los taninos hidrolizables, incluyendo las punicalaginas (Gil y col., 2000; Hagerman y col., 1998). Con respecto al análisis de la fase desproteinizada (permeato) los resultados revelaron que el ácido elágico no se une a las proteínas pues su contenido permaneció estable: 9.95 mg/L a día 1 de almacenamiento y 10.87 mg/L a día 28. Sin embargo no se detectó contenido alguno de punicalaginas lo cual indica que tienen una gran afinidad por las proteínas lácteas. Por tanto, únicamente el ácido elágico permanece en su forma libre en el yogur.

En la **Tabla 27** se presenta el contenido de las antocianinas previamente identificadas en zumo de granada y en los yogures y permeatos a lo largo del almacenamiento. Las antocianinas están asociadas con la prevención de ECV, obesidad y diabetes (Jurenka, 2008). Se puede decir, a diferencia de los otros compuestos fenólicos identificados, que el zumo de granada es extremadamente rico en antocianinas (641.6 mg/L). Cabe destacar que el contenido en antocininas determinado por el método cromatográfico aporta valores muy superiores a los del método colorimétrico de Wrolstad (1993), se debe tener en cuenta que los resultados de la cromatografía son mucho más fiables por permitir la identificación de compuestos individuales y su cuantificación, mientras que el método colorimétrico nos lleva a expresar la concentración final en forma de equivalentes de cianidin-3-glucósido. Ambos resultados son de interés dado que muchos autores utilizan el método colorimétrio para cuantificar antocianos y ésto nos permite comparar nuestros resultados con referencias anteriores. La antocianina más abundante en PGJ fue cianidin-3-*O*-glucosido, lo cual es característico de la variedad 'Mollar de Elche' (Mena y col., 2011). En cambio cuantitativamente en los yogures la distribución relativa de las antocianinas fue ligeramente distinta a la del PGJ. En los yogures la antocianina más abundante fue cianidin-3,5-*O*-diglucosido, seguida de cianidin-3-*O*-glucosido (durante todos el almacenamiento). Está demostrado que el proceso de fermentación del PGJ causa pérdidas en el contenido de antocianinas

Tabla 27. Contenido en antocianinas individuales (mg/L) en zumo de granada, yogur con zumo de granada y permeato de yogur (media ± error estándar).

	Antocianina							
Muestra	**Dp 3,5-diglc**	**Cy 3,5-diglc**	**Dp 3-glc**	**Pg 3,5-diglc**	**Cy 3-glc**	**Pg 3-glc**	**Cy-pent**	**Antocianinas Totales**
PGJ	78.41±0.78	190.03±1.88	62.85±0.65	21.55±0.25	221.00±2.20	64.65±0.65	3.00±0.10	641.48±1.25
PGY d1	61.80±0.60	162.70±1.60	49.35±0.45	16.45±0.15	125.65±1.25	24.45±0.25	1.75±0.05	442.15±3.05
PGY d14	59.85±0.55	149.65±1.45	43.45±0.45	15.75±0.15	110.40±1.10	23.05±0.25	1.55±0.05	403.70±0.10
PGY d28	31.40±0.30	103.10±1.00	33.10±0.30	9.85±0.25	80.60±0.80	16.35±0.15	1.25±0.05	275.65±0.85
Permeato d1	47.55±1.45	0.00±0.00	0.00±0.00	3.45±0.05	12.85±015	3.65±0.05	0.00±0.00	67.50±1.20
Permeato d28	16.85±1.15	0.00±0.00	0.00±0.00	1.25±0.05	7.25±0.15	2.05±0.05	0.00±0.00	27.40±0.90

Abreviaciones: Dp-3,5-diglc, delfinidin-3-5-*O*-diglucosido; Cy-3,5-diglc, cianidin-3,5-*O*-diglucosido; Dp-3-glc, delfinidin-3-*O*-glucosido; Pg-3,5-diglc, pelargonidin-3,5-*O*-diglucosido; Cy-3-glc, cianidin-3-*O*-glucosido; Pg-3-glc, pelargonidin-3-*O*-glucosido; Cy-pent, cianidin-pentosido; PGJ, zumo de granada; PGY, yogur con zumo de granada. Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

(Mena y col., 2012). La estructura de la antocianina influencia la estabilidad, los monoglucósidos son menos estables que los diglucosidos (Mena y col., 2012). En el presente estudio las antocianinas menos estables a la fermentación fueron cianidin-3-*O*-glucosido y pelargonidin-3-*O*-glucosido mientras que cianidin-3,5-*O*-diglucosido resultó ser la más estable. Con respecto a la estabilidad de las antocianinas individuales en los yogures (**Figura 11**), se observaron pérdidas significativas durante su almacenamiento. Se sabe que las antocianinas siguen reacciones de degradación de primer orden (Giusti y Wrolstad, 1996; Wang y Xu, 2007).

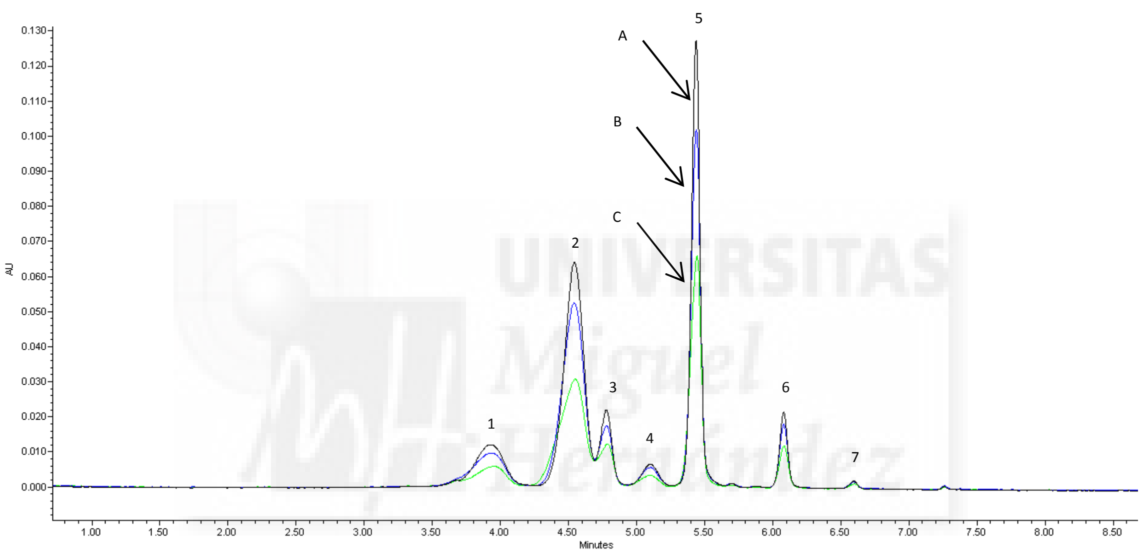

Figura 11. Cromatogramas del perfil de antocianinas en yogur con granada tras 1 (A), 14 (B) y 28 (C) días de almacenamiento.

Resultados de los permeatos (**Tabla 27**) revelaron que en el caso de las antocianinas muchas permanecieron unidas a proteínas: cianidin-3-*O*-glucosido, la principal antocianina en PGJ, no se detectó en el permeato al igual que definidin-3-*O*-glucosido y cianidin-pentosido. Delfinidin-3,5-*O*-diglucosido resultó ser la antocianina más estable mostrando el menor ratio de interacción con las proteínas. En general la afinidad de las antocianinas por las proteínas es muy elevada pues a día 1 de almacenamiento el 84.73% de las antocianinas permanecieron unidas a las proteínas, porcentaje que aumentó hasta un 90.06% a día 28. Xiao y col., (2011) señalaron que

los flavonoides monoglucosidos presentan mayor afinidad para unirse a las proteínas lácteas que sus formas poliglucosiladas. Además se ha demostrado que la AA de los polifenoles disminuye a medida que aumentan las interacciones entre los complejos proteína-polifenol (Xiao y col., 2011).

4.2.2.9. Contenido en fenoles, flavonoides y antocianos totales en yogur enriquecido en zumo de granada y grado de interacción proteína-polifenol

El yogur con granada contenía un 40% de PGJ y presentó 241.44 mg GAE/L, lo que se traduce en un 85.35% de lo esperado. El primer día de almacenamiento el contenido en fenoles totales en los permeatos fue de 111.92 mg GAE/L lo que significa que cerca del 54% de las sustancias fenólicas totales permanecían en la fase proteica interaccionando con las proteínas. Al final del periodo de almacenamiento este porcentaje disminuyó (40.94%), siendo 142.660 mg GAE/L y 84.84 mg GAE/L el contenido en fenoles totales del yogur con granada y el permeato, respectivamente. Durante el almacenamiento los yogures con granada sufrieron las mayores pérdidas de TPC (40.94%) ($p<0.05$). La estabilidad de los pigmentos y los compuestos fenólicos en los yogures se ve afectada por la temperatura de almacenamiento, pH, contenido en fenoles, grasa y el tipo de cultivo iniciador utilizado (Ścibisz y col., 2012; Wallace y Giusti, 2008).

Teniendo en cuenta el contenido en flavonoides totales del PGJ (75.50 mg RE/100 g), el determinado en los yogures fue mayor de lo esperado (34.13 mg RE/100 g). Los permeatos retuvieron el 65.78% y 90% de los flavonoides totales en el día 1 y 28 de almacenamiento, respectivamente. Por lo que por medios colorimétricos observamos que la afinidad de los flavonoides por las proteínas es menor que la de otros compuestos fenólicos. Es interesante destacar que aunque el yogur no es fuente de compuestos fenólicos (O'Conell y Fox, 2001) el yogur control presentó unos valores de TPC y TFC de 39.08 mg GAE/L y 3.78 mg RE/100 g a día 1 de almacenamiento. A lo largo del almacenamiento el contenido en flavonoides totales disminuyó en todas las muestras (**Figura 12**), aunque sólo de forma significativa en los yogures con granada.

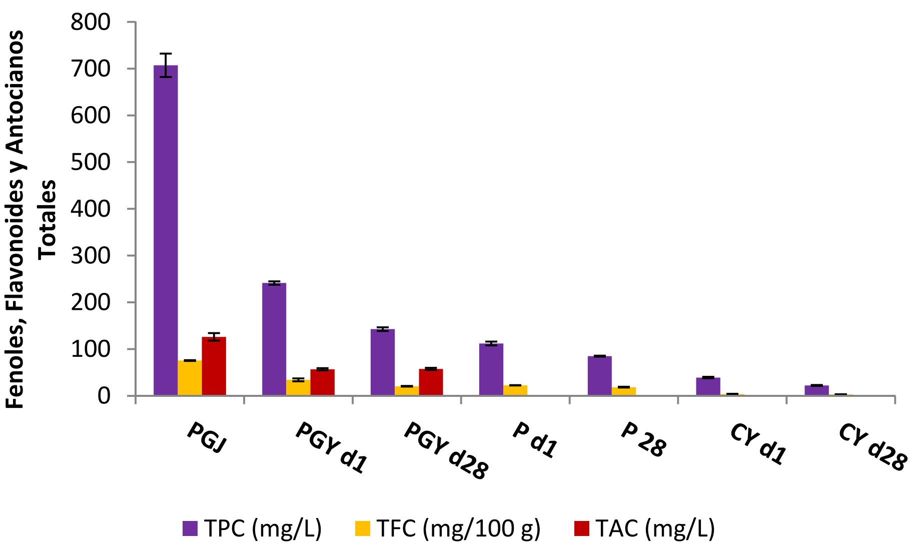

Figura 12. Contenido en fenoles totales (TPC; mg GAE/L), flavonoides totales (TFC; mg RE/100 g) y antocianos totales (TAC; mg equivalentes cianidin-3-glucosido/L) del zumo de granada, yogures y permeatos durante 28 días de almacenamiento a 4 ^{o}C.

El contenido en antocianos totales (determinado por métodos colorimétricos) del yogur con granada no se vio afectado por el tiempo de almacenamiento ($p>0.05$) lo cual no se correlaciona con los resultados obtenidos por cromatografía. Sin embargo sí están correlacionados con los obtenidos por las determinaciones de color del espacio CIE*L*a*b** (**Tabla 28**). Durante el periodo de almacenamiento la coordenada *a** (rojez) aumentó y la *b** (amarilla) disminuyó ($p<0.05$), lo que quiere decir que el color rojo de los yogures se refuerza. Además, aunque de forma no significativa, la coordenada *L** (luminosidad) disminuye durante el almacenamiento signo de estabilización de los pigmentos. Esta estabilización puede ser debida a co-pigmentación inter-/intra-molecular y a reacciones de auto-asociación (Wallace y Giusti, 2008). Finalmente, en los permeatos no se detectó contenido en antocianos por métodos colorimétricos cuantitativos.

Tabla 28. Características de color de los yogures durante 28 días de almacenamiento en refrigeración (media ± error estándar).

Tipo de yogur	***L****	***a****	***b****
Control	84.61 ± 1.16^{b}	-2.73 ± 0.06^{a}	5.27 ± 0.17^{c}
PGY d1	66.65 ± 0.41^{a}	5.61 ± 0.10^{b}	4.93 ± 0.11^{b}
PGY d14	65.95 ± 0.05^{a}	5.78 ± 0.02^{b}	$4.83\pm0.05^{a,b}$
PGY d28	65.24 ± 0.52^{a}	6.26 ± 0.04^{c}	4.56 ± 0.04^{a}

Abreviaciones: PGY, yogur con zumo de granada
Valores en la misma columna con distinta letra difieren significativamente ($p<0.05$).

4.2.2.10. Actividad antioxidante del yogur enriquecido en zumo de granada y grado de interacción proteína-polifenol

La actividad antioxidante se determinó mediante dos métodos: el ensayo del secuestro del radical DPPH y la determinación del poder de reducción del hierro (FRAP) y los resultados de dichos ensayos se presentan en la **Tabla 29**.

Los yogures con granada mostraron la mayor actividad secuestrante de radicales y ello se vio reflejado en su menor valor de IC_{50}. Teniendo en cuenta los resultados obtenidos para el PGJ y para los yogures control es posible que haya tenido lugar una acción sinergística al producirse el yogur enriquecido, de hecho el valor de IC_{50} del mix de leche y PGJ (previo a la fermentación) fue de 4.72. A lo largo del almacenamiento la actividad secuestrante de radicales no se vio disminuida de forma significativa, en este punto resultados sobre el contenido en compuestos fenólicos totales indicaron que tanto TPC como TFC disminuyeron significativamente (**Figura 12**), únicamente TAC se mantuvo estable tras los 28 días. De forma que es posible que sean los antocianos los compuestos fenólicos que le proporcionan dicha actividad secuestrante al yogur, aunque estadísticamente no estuvo correlacionado (**Tabla 29**).

Según los resultados del FRAP el PGJ mostró con diferencia el mayor poder de reducción de hierro ($p<0.05$). En PGJ el FRAP estuvo correlacionado con el contenido en TPC y TAC (**Tabla 30**). A día 1 de almacenamiento los resultados obtenidos en los yogures con granada corresponden con cerca del 40% de los del PGJ; teniendo en cuenta que en este caso los yogures control apenas presentaron actividad se puede concluir que la AA de los yogures enriquecidos en este caso viene dada por el PGJ.

Tabla 29a. Actividad antioxidante del zumo de granada, yogures y permeatos durante el almacenamiento a 4 °C a diferentes concentraciones (A = 2.5 g/100 mL, B = 5 g/100 mL, C = 7.5 g/100 mL, D = 10 g/100 mL) (media ± error estándar).

Muestra	**Día**	**DPPH•**	**FRAP**			
		IC_{50}[1]	**TEAC[2] (mM Trolox/L)**			
			A	**B**	**C**	**D**
PGJ		10.45[b]	0.254 ± 0.006[aD]	0.479 ± 0.001[bE]	0.710 ± 0.011[cD]	0.870 ± 0.011[dD]
PGY	1	2.51[a]	0.090 ± 0.003[aC]	0.132 ± 0.002[a,bD]	0.215 ± 0.036[b,cC]	0.279 ± 0.030[cC]
	28	2.45[a]	0.060 ± 0.002[aB]	0.115 ± 0.001[bC]	0.163 ± 0.002[cB,C]	0.202 ± 0.009[dB]
Permeato	1	70.62[c]	0.061 ± 0.001[aB]	0.111 ± 0.001[bC]	0.164 ± 0.001[cB,C]	0.208 ± 0.004[dB]
	28	85.46[c]	0.049 ± 0.001[aB]	0.089 ± 0.003[bB]	0.132 ± 0.001[cB]	0.162 ± 0.002[dB]
CY	1	5.38[b]	0.023 ± 0.002[aA]	0.036 ± 0.000[bA]	0.048 ± 0.000[cA]	0.062 ± 0.001[dA]
	28	6.96[b]	0.022 ± 0.000[aA]	0.028 ± 0.004[a,bA]	0.044 ± 0.000[b,cA]	0.054 ± 0.004[cA]

Abreviaciones: PGJ, zumo de granada; PGY, yogur con granada; CY, yogur control.
[1]IC_{50}, concentración (g/100 mL) para un 50% de inhibición. [2]TEAC, capacidad antioxidante en equivalentes de Trolox.
Valores en la misma línea con distinta letra minúscula difieren significativamente ($p<0.05$).
Valores en la misma columna con distinta letra mayúscula difieren significativamente ($p<0.05$).

Tabla 29b. Actividad antioxidante relativa del zumo de granada, yogures y permeato.

Muestra	DPPH•	FRAP
PGJ	++	++++
PGY	++++	++
Permeato	-	++
CY	+++	+

Ver Tabla 29a para abreviaciones.

De forma simplificada en la **Tabla 29b** se puede intuir cómo de diferente se comporta el mismo compuesto ante distintos métodos de medición de la AA. A lo largo del almacenamiento únicamente los yogures con granada disminuyeron su poder reductor del hierro ($p<0.05$) presentando al final del almacenamiento valores similares a los del permeato.

Los permeatos no presentaron actividad secuestrante de radicales y su poder reductor del hierro fue similar al presentado por los yogures enriquecidos. Los permeatos, al igual que en PGJ, están libres de proteínas con lo que su potencial antioxidante mayoritariamente se le atribuye a los compuestos fenólicos. De hecho los permeatos están fuertemente correlacionados con su contenido en fenoles y flavonoides totales (**Tabla 30**). En cambio el yogur es una matriz compleja donde no se puede establecer con seguridad si su potencial antioxidante es debido únicamente a su contenido en compuestos fenólicos o a una causa multifactorial. Del presente estudio se deriva que la interacción de las proteínas lácteas con los compuestos fenólicos del PGJ no afecta a la actividad antioxidante de los yogures.

Tabla 30. Matriz de correlación entre los métodos de actividad antioxidante y el contenido en fenoles, flavonoides y antocianos totales del zumo de granada, yogures y permeatos.

Muestra	Método	TPC	TFC	TAC
PGJ	DPPH•	0.98	0.10	1
	FRAP	0.99	0.45	0.92
PGY	DPPH•	0.64	0.88	0.24
	FRAP	0.89	0.77	0.72
Permeatos	DPPH•	0.90	0.99	---
	FRAP	0.99	0.98	---
CY	DPPH•	0.57	0.01	---
	FRAP	0.84	0.42	---

Abreviaciones: TPC, contenido en fenoles totales; TFC, contenido en flavonoides totales; TAC, contenido en antocianos totales; PGJ, zumo de granada; PGY, yogur con granada; CY, yogur control.
El valor *r* de correlación es dado. Todas las correlaciones fueron significativas a $p<0.05$.

4.2. BLOQUE II: ESTUDIO DE ÁCIDOS GRASOS CONJUGADOS COMO INGREDIENTES FUNCIONALES Y EVALUACIÓN DEL CONTENIDO EN ÁCIDO LINOLEICO CONJUGADO (CLA) EN LECHES FERMENTADAS

La obesidad es uno de los principales problemas de salud siendo el quinto factor de riesgo de defunción a nivel mundial (WHO, 2013b). En consecuencia el conocimiento de los mecanismos que subyacen el control del apetito es una potente herramienta para dirigir la epidemia de la obesidad (Vincent y le Roux, 2007). La aplicación de este conocimiento en el desarrollo de ingredientes funcionales que promuevan la pérdida de peso es un concepto prometedor en la lucha contra la obesidad, de ahí el interés de la presente Tesis en el estudio del ácido linoleico conjugado (CLA) como ingrediente funcional con potencial anti-obesidad.

El proceso de revisión sobre los ácidos grasos conjugados, y en concreto CLA, como ingredientes funcionales con efecto anti-obesidad se incluye en la publicación de la revista *Critical Reviews in Food Science and Nutrition* (2013, 53(9): 929-942). La evaluación del contenido de CLA en leches fermentadas comerciales se encuentra bajo revisión en la revista *LWT - Food Science and Technology* y la determinación del contenido de CLA en leche de cabra fermentada se encuentra bajo revisión en la revista *International Journal of Dairy Technology*.

4.2.1. ÁCIDOS GRASOS CONJUGADOS COMO INGREDIENTES FUNCIONALES CON POTENCIAL EFECTO ANTIOBESIDAD

Como resultado de la revisión bibliográfica se desprende que el estudio sobre el potencial efecto de los ácidos grasos en la reducción de la grasa corporal se ha centrado principalmente en los AGPI *n*-3 (EPA y DHA) y ácido linoleico conjugado (CLA). Numerosos estudios prueban su efectividad en modelos animales si bien los estudios en humanos mostraron diversos resultados no aclarándose de forma inequívoca su efecto positivo. Puesto que en la presente Tesis se continuó con el estudio del CLA en leches fermentadas a continuación se presenta un resumen de los resultados encontrados para los ácidos grasos conjugados.

Los ácidos grasos conjugados (CFAs) son una mezcla de isómeros posicionales y geométricos de los ácidos grasos poliinsaturados con dobles enlaces conjugados. Como anteriormente se ha explicado (*Véase* sección **1.7.2**. **Implicación del ácido linoleico conjugado en la salud**) el CLA es la forma conjugada del ácido linoleico. En estudios animales CLA ha mostrado reducir la grasa corporal y aumentar la masa magra en varias especies como ratas, ratones y cerdos (Azain y col., 2000; DeLany y West, 2000; Meadus y col., 2002). Jahreis y col., (2000) atribuyeron estos efectos a la reducción de la actividad de la lipoproteinalipasa (LPL) y a un aumento de la enzima carnitina palmitoiltransferasa (CPT) (asociada a la β-oxidación de los lípidos).

De entre los 28 isómeros posibles del CLA sólo se conoce bioactividad en dos de ellos: el isómero *trans*-10, *cis*-12-CLA, relacionado con su efecto anti-obesidad, además de anti-carcinogénico y antidiabético, y el isómero *cis*-9, *trans*-12-CLA relacionado con su efecto anti-carcinogénico (Nagao y Yanagita, 2005). Estudios en humanos han probado que la suplementación con mix de isómeros de CLA reduce el peso corporal (Blankson y col., 2000; Thom y col., 2001) y el porcentaje de grasa corporal (Smedman y Vessby, 2001). Sin embargo revisiones realizadas por otros autores (Larsen y col., 2003) concluyeron que la evidencia en la reducción del peso corporal es baja en estudios en humanos con duraciones inferiores a 6 meses o directamente que no la hay en humanos aunque sí en animales (Silveira y col., 2007).

Otros CFAs distintos al CLA han sido estudiados por su efecto anti-obesidad. Tsuzuki y col., (2005, 2006) diseñaron ácido eicosapentanoico conjugado (CEPA) y ácido docosahexanoico conjugado (CDHA) mediante isomerización alcalina con la hipótesis de que la combinación de los dobles enlaces conjugados junto con la estructura altamente insaturada podría tener mayor efecto anti-obesidad. La administración a corto plazo de CEPA y CDHA en animales suprimía la acumulación lipídica en hígado y tejido adiposo mucho más que CLA, EPA y DHA.

4.2.2. CLA EN LECHES FERMENTADAS COMERCIALES

La distribución media de las fracciones de ácidos grasos saturados (SFA), monoinsaturados (MUFA) y poliinsaturados (PUFA) de las leches fermentadas (LF) analizadas (**Tabla 31**) está en el rango de valores presentados en la revisión realizada

Tabla 31. Composición porcentual en ácidos grasos de las leches fermentadas (media ± error estándar).

Tipo de LF	Ácidos grasos, % del total de ácidos grasos									
	C16:0	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**c9 t11 CLA**	**t10 c12 CLA**	**SFA**	**MUFA**	**PUFA**	**ΣCLA**
B1	31.87±0.79	1.46±0.05	12.36±0.25	25.60±0.64	2.87±0.09	0.57±0.00	0.19±0.00	68.67±0.82	27.06±0.68	4.27±0.14	0.77±0.01
B2	32.41±0.11	1.52±0.05	11.94±0.36	24.61±0.10	2.81±0.10	0.56±0.06	0.22±0.00	69.60±0.11	26.13±0.15	4.27±0.04	0.77±0.07
B3	31.64±0.16	1.41±0.05	12.20±0.03	24.69±0.02	2.89±0.04	0.55±0.02	0.20±0.00	69.49±0.22	26.10±0.03	4.41±0.25	0.75±0.03
Lc1	32.50±0.81	1.52±0.04	12.37±0.30	25.68±0.59	2.51±0.07	0.67±0.02	0.24±0.00	68.66±0.71	27.20±0.63	4.14±0.07	0.91±0.03
Lc2	33.47±0.24	1.59±0.03	12.22±0.14	24.37±0.55	2.56±0.04	0.52±0.00	0.21±0.00	70.07±0.57	25.96±0.58	3.98±0.01	0.73±0.00
Lc3	32.15±0.23	1.52±0.02	12.01±0.45	24.60±0.48	2.43±0.08	0.63±0.02	0.25±0.00	69.93±0.60	26.12±0.50	3.96±0.11	0.88±0.03
Y1	29.07±0.74	1.26±0.01	13.63±0.42	28.29±0.98	4.00±0.23	0.59±0.01	0.23±0.01	64.58±1.65	29.55±0.97	5.87±0.69	0.82±0.02
Y2	32.87±0.58	1.59±0.01	11.78±0.22	25.22±0.98	2.64±0.10	0.56±0.00	0.21±0.01	69.02±1.11	26.81±0.99	4.17±0.12	0.78±0.01
Y3	30.54±0.50	1.31±0.00	13.07±0.08	26.43±0.22	3.26±0.04	0.60±0.01	0.24±0.00	67.17±0.23	27.74±0.23	5.09±0.49	0.84±0.01
Y4	33.43±0.72	1.64±0.02	11.42±0.17	25.57±0.04	2.47±0.16	0.59±0.02	0.20±0.00	68.84±0.11	27.21±0.06	3.95±0.05	0.79±0.03
G1	32.75±1.32	1.63±0.12	11.13±1.12	24.38±1.28	2.59±0.58	0.61±0.06	0.19±0.01	69.96±1.61	26.01±1.16	4.02±0.46	0.79±0.05
G2	30.29±0.09	1.52±0.01	11.19±0.02	25.34±0.19	2.11±0.06	0.98±0.00	0.20±0.01	68.58±0.25	26.86±0.21	4.55±0.05	1.18±0.02
G3	33.22±1.17	1.66±0.10	11.29±1.36	24.48±1.53	2.61±0.58	0.63±0.06	0.19±0.01	69.95±1.86	26.14±1.43	3.92±0.44	0.81±0.06
G4	32.62±0.07	1.63±0.01	11.31±0.04	24.61±0.13	2.21±0.02	0.63±0.00	0.21±0.00	70.09±0.15	26.24±0.12	3.67±0.03	0.84±0.00
B1-L	32.15±0.21	1.44±0.18	12.83±0.14	25.83±0.62	3.53±0.15	0.49±0.04	0.36±0.00	67.67±1.15	27.27±0.48	5.06±0.29	0.85±0.06
B2-L	30.73±0.30	1.54±0.11	13.62±0.31	23.73±0.38	6.19±0.24	n.d.	n.d.	64.85±0.24	23.73±0.32	6.42±0.13	n.d.
B3-L	31.15±0.11	1.59±0.04	12.82±0.25	24.17±0.24	3.32±0.18	0.50±0.11	0.96±0.02	68.98±0.18	25.76±0.41	5.26±0.24	1.46±0.04
Lc1-L	32.48±0.15	1.37±0.08	14.36±0.08	26.93±0.27	4.03±0.42	0.54±0.08	0.34±0.01	66.11±0.95	28.29±0.44	5.60±0.15	0.88±0.01
Lc2-L	30.43±0.14	1.41±0.04	16.78±0.40	21.17±0.75	4.96±0.31	n.d.	n.d.	69.09±0.97	22.58±0.65	8.32±0.35	n.d.
Lc3-L	30.75±0.28	1.14±0.08	21.82±0.45	19.51±0.71	4.23±0.15	n.d.	1.46±0.01	73.66±1.15	20.65±0.28	5.69±0.44	1.46±0.01
Y1-L	30.91±0.41	1.42±0.14	12.08±0.25	24.08±0.41	3.15±0.12	0.50±0.05	0.24±0.02	69.94±0.35	25.51±0.27	4.55±0.47	0.74±0.02
Y2-L	30.26±0.54	1.23±0.12	16.45±0.31	19.98±0.40	4.53±0.39	n.d.	n.d.	63.77±0.61	21.21±0.38	6.02±0.68	n.d.
Y3-L	33.03±0.19	1.11±0.20	14.03±0.14	24.59±0.84	4.72±0.24	0.75±0.02	1.35±0.02	68.59±0.64	24.59±0.39	6.82±0.14	2.11±0.01
G1-L	31.17±0.24	1.51±0.05	10.24±0.08	22.21±0.16	2.42±0.11	0.45±0.01	0.20±0.00	72.62±0.18	23.72±0.44	3.66±0.31	0.64±0.04

Abreviaciones: LF, leche fermentada; t, *trans*; c, *cis*, CLA, ácido linoleico conjugado; SFA, ácidos grasos saturados; MUFA, ácidos grasos monoinsaturados; PUFA, ácidos grasos poliinsaturados; n.d., no detectado; B, LF con los cultivos tradicionales del yogur (CTY) (*L. bulgaricus* y *S. thermophilus*) y *Bifidobacterium* spp.; Lc, LF con los CTY y *L. casei*; Y, LF con los CTY; G, LF con los CTY al estilo griego; L detrás de cada tipo de LF representa el producto bajo en grasa.
SFA: suma de C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C19:0, C20:0 y C21:0; MUFA: suma de C16:1 y C18:1; PUFA: suma de todos los C18:2, C18:3 y C20:5.

por Jensen y col., (2002). La contribución del CLA en el perfil total de ácidos grasos fue de 0.83% de media (0.50% de isómero *cis*-9, *trans*-11-CLA y 0.33% de isómero *trans*-10,-*cis*-12-CLA), lo cual está en concordancia con lo estudiado previamente por otros autores (Fritsche y Steinhart, 1998; Serafeimidou y col., 2012).

Como se puede observar en la Tabla XX en la mayoría de muestras analizadas el isómero mayoritario es el *cis*-9, *trans*-11-CLA, únicamente en ciertos tipos de LF bajas en grasa el isómero *trans*-10,-*cis*-12-CLA es el mayoritario aunque esto puede considerarse como irrelevante debido al bajo contenido en grasa que presentan (<0.5%) y a los problemas de detección de ácidos grasos minoritarios en este tipo de muestras.

Ni el contenido en grasa de las LF ni el tipo de cultivo iniciador afectó al contenido total de CLA (p>0.05), cuando los resultados son expresados como % del total de ácidos grasos (**Tabla 31**). Únicamente el isómero *cis*-9, *trans*-11-CLA individualmente se vio afectado, siendo su contenido significativamente mayor en las LF con los CTY al estilo griego con alto contenido en grasa (>8%) (G) con respecto a las LF bajas en grasa (<0.5%) (todos los tipos). En la **Tabla 32** se presentan los resultados en mg/100 mL de leche

Tabla 32. Contenido en CLA (mg/100 g muestra) y grasa (g/100 g muestra) de las distintas leches fermentadas.

Tipo de LF[1]	CLA total (mg/100 g)		Grasa (g/100 g)[2]
	Media	E. E.	
B1	24.81	0.73	3.70
B2	22.46	2.38	3.50
B3	25.63	0.82	3.60
Lc1	22.19	2.35	2.60
Lc2	11.95	0.80	1.60
Lc3	10.42	0.27	1.40
Y1	21.70	0.71	2.90
Y2	20.87	1.32	3.60
Y3	12.82	0.56	2.20
Y4	23.44	0.60	3.10
G1	64.47	1.02	10.00
G2	93.33	1.95	8.70
G3	73.32	1.47	9.10

G4	53.78	0.27	9.00
B1-L	3.35	0.03	0.40
B2-L	n.d.	---	0.10
B3-L	1.64	0.04	0.40
Lc1-L	3.96	0.11	0.10
Lc2-L	n.d.	---	0.20
Lc3-L	1.10	0.05	0.50
Y1-L	2.94	0.14	0.40
Y2-L	n.d.	---	0.10
Y3-L	2.41	0.20	0.10
G1-L	1.92	0.16	2.90

[1]Ver Tabla XX para abreviaciones.
[2]Información dada en el etiquetado del producto.
E. E., error estándar.

Cuando el análisis estadístico es llevado a cabo en mg/100 g de muestra el contenido en CLA se ve afectado por la cantidad de grasa de la LF (Tabla XX). A mayor contenido en grasa, mayor cantidad de CLA ($p<0.05$). La ingesta diaria estimada de CLA varía entre 200 – 1000 mg al día (Van Wijlen y Colombani, 2010), aunque según Ip y col., (1994) esta ingesta no sería suficiente para alcanzar un efecto beneficioso pues establecen que un adulto con un peso medio de 70 kg debería de ingerir 3000 mg de CLA/día. De los resultados desprendidos en el presente estudio el consumo de una ración (125 g) de LF con alto contenido (>0.8%), medio (1.4%-4%) y bajo (<0.5%) en grasa contribuye al 2.97%, 0.82% y 0.11%, respectivamente, de las recomendaciones diarias para conseguir un efecto beneficioso en la salud.

4.2.3. CLA EN LECHES FERMENTADAS DE CABRA

4.2.3.1. Composición química de la leche de cabra

La leche cruda de cabra presentó la siguiente composición química: 6.38% de grasa, 4.31% de proteína, 3.41% de caseína, 4.27% de lactosa, 15.36% de materia seca, 0.69% de ácidos grasos libres (expresados como g ácido oleico/100 g de grasa) y un pH de 6.60. El contenido en grasa de la leche fue superior al revisado en la literatura (Boyazoglu y Morand-Fehr 2001; Tamime y col., 2011), sin embargo la composición química de cualquier tipo de leche fresca varía a lo largo del tiempo en función de factores tales como la raza, animal individual, dieta y alimentación, condiciones

ambientales y locales, período de lactación, estado sanitario, etc. (Slačanac y col., 2010).

4.2.3.2. Proceso de fermentación

Ni el empleo de aceite de girasol hidrolizado (HSO) como fuente de ácido linoleico libre ni el uso de Tonalin® afectó al pH de las leches fermentadas durante el proceso de fermentación ($p>0.05$).

Tabla 33. Perfil de ácidos grasos (% del total de ácidos grasos) de la leche cruda de cabra y de las distintas leches control fermentadas (yogur y *L. casei*) (transcurridas 24h de elaboración).

Ácido graso	Leche cruda de cabra		Yogur		*L. casei*	
	Media	E. E.	Media	E. E.	Media	E. E.
C4:0	0.69	0.09	0.56	0.13	0.68	0.05
C6:0	1.74	0.24	1.52	0.07	1.82	0.32
C8:0	2.56	0.46	2.26	0.13	2.71	0.57
C10:0	8.46	1.12	7.64	0.32	8.70	1.98
C12:0	3.78	0.16	3.64	0.19	3.98	0.68
C14:0	9.11	0.33	8.85	0.70	8.83	0.79
C15:0	0.78	0.22	0.74	0.19	0.76	0.19
C16:0	26.61	0.37	27.09	0.20	26.31	0.90
C16:1	1.22	0.14	1.20	0.14	0.93	0.63
C18:0	13.27	0.12	14.03	0.93	13.36	0.56
C18:1	26.93	1.01	27.62	0.54	27.01	1.22
C18:2	2.79	0.15	2.86	0.24	2.74	0.13
C18:3	0.08	0.04	0.08	0.03	0.16	0.12
C18:2 *c*9,*t*11-CLA	0.81[a]	0.03	0.99[b]	0.04	1.00[b]	0.14
C18:2 *t*10,*c*12-CLA	n.d.	---	n.d.	---	n.d.	---
C20:0	0.25	0.09	0.27	0.05	0.28	0.08
C20:1	0.74	0.08	0.33	0.20	0.58	0.05
SFA	67.26	0.96	66.60	0.59	67.45	1.90
MUFA	28.89	1.07	29.15	0.92	28.52	1.71
PUFA	3.68	0.18	3.93	0.27	3.90	0.28
ΣC18	43.88	1.00	45.58	1.00	44.27	1.83
ΣCLA	0.81	0.03	0.99	0.02	1.00	0.07

Abreviaciones: E. E., error estándar; n.d., no detectado; t, *trans*; c, *cis*; CLA, ácido linoleico conjugado; SFA, ácidos grasos saturados; MUFA, ácidos grasos monoinsaturados, PUFA, ácidos grasos poliinsaturados.
SFA: suma de C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 y C20:0; MUFA: suma de C16:1, C18:1 y C20:1; PUFA: suma de todos los C18:2 y C18:3.
Valores en la misma línea con distinta letra difieren significativamente ($p<0.05$).

El efecto del empleo de los distintos cultivos iniciadores sobre el perfil de ácidos grasos de la leche cruda de cabra se presenta en la **Tabla 33**. El empleo tanto de los CTY como *L. casei* aumentó el contenido del isómero *cis*-9, *trans*-11-CLA. En este caso como la leche cruda de cabra no estaba suplementada dicho incremento se debe únicamente al proceso de fermentación. En las muestras no suplementadas no se detectó presencia del isómero *trans*-10, *cis*-12-CLA (**Tabla 33**).

4.2.3.3. Evolución del perfil de ácidos grasos las leches fermentadas durante el almacenamiento

En primer lugar se realizaron ensayos preliminares con **aceite de girasol sin hidrolizar**. Tanto en las LF con los CTY como con *L. casei* el contenido en ácido linoleico y en CLA permaneció inalterado tanto al final de la fermentación como a lo largo del almacenamiento. Por lo tanto, las bacterias lácticas en estudio no son capaces producir CLA a partir del aceite sin hidrolizar. Estos resultados coinciden con los publicados por Xu y col., (2004) y Gorissen y col., (2012). Además, para una mejor comprensión del comportamiento de las bacterias lácticas se evaluó la ratio de hidrólisis tanto del aceite de girasol nativo (SO) como hidrolizado (HSO). Como podemos observar en la Tabla XX el HSO estaba completamente hidrolizado. En cuanto a Tonalín® la ficha técnica del fabricante indicaba en su composición que estaba más del 98% en forma de triglicéridos.

Tabla 34. Porcentaje de hidrólisis del aceite de girasol.

%	HSO (%)	SO (%)
Ácidos grasos libres	60.08	0
Monoglicéridos	19.12	4.72
Diglicéridos	19.96	9.88
Glicerol	0.84	0
Triglicéridos	0	85.4

Abreviaciones: HSO, aceite de girasol hidrolizado; SO, aceite de girasol (nativo).

En cuanto al **yogur**, la suplementación con HSO o Tonalin® afectó dramáticamente su perfil de ácidos grasos (**Tabla 35a**). El HSO contenía un 61.68% de ácido linoleico con lo que el contenido de dicho ácido graso en los yogures suplementados fue superior

($p<0.05$). Por el mismo motivo los yogures con Tonalin® presentaron mayor concentración ($p<0.05$) de los isómeros de CLA. En la **Tabla 33** se ha podido observar el aumento de los niveles de CLA a través del proceso de fermentación. La suplementación con HSO se llevó a cabo para evaluar la posible aceleración en la producción de CLA, lo cual ha sido analizado en estudios anteriores (Colakoglu y Gursoy 2011; Xu y col., 2005). En el presente estudio se comprobó que los CTY no aumentaron la concentración del isómero *cis*-9, *trans*-11-CLA; sin embargo sí fueron capaces de producir el isómero *trans*-10, *cis*-12-CLA a partir de ácido linoleico libre. El alto contenido en ácidos grasos libres de la leche de cabra (0.69% del total de la grasa) junto con el elevado contenido de ácido linoleico suplementado pudieron haber actuado como potenciadores o iniciadores de la isomerización del CLA (Gorissen y col., 2012). La concentración máxima de CLA en los yogures suplementados con HSO se detectó a día 28 de almacenamiento (1.02% y 0.18% (del total de ácidos grasos) para los isómeros *cis*-9, *trans*-11-CLA y *trans*-10, *cis*-12-CLA, respectivamente).

Tabla 35a. Contenido en C18s y principales grupos de ácidos grasos (% del total de ácidos grasos) en yogures de leche de cabra en función de la fuente lipídica y el tiempo de almacenamiento en refrigeración (media ± error estándar).

	Tiempo (días)				
	0	**1**	**14**	**28**	**35**
C18:2					
Control	2.96±0.33	2.88±0.05	2.73±0.01	2.71±0.04	2.72±0.11
Hidrolizado	10.96±0.97	10.34±0.12	9.50±0.21	9.21±0.14	11.25±0.03
Tonalin®	2.76±0.34	2.67±0.02	2.65±0.05	2.73±0.05	2.52±0.01
C18:2 *c*9,*t*11-CLA					
Control	0.97±0.02	0.98±0.04	0.96±0.01	0.93±0.03	0.93±0.02
Hydrolyzed	0.96±0.05	1.00±0.06	0.94±0.03	1.02±0.01	0.93±0.03
Tonalin®	1.39±0.27	1.32±0.11	1.27±0.04	1.27±0.04	1.31±0.09
C18:2 *t*10,*c*12-CLA					
Control	n.d.	n.d.	n.d.	n.d.	n.d.
Hidrolizado	0.14±0.01	0.08±0.03	0.11±0.05	0.18±0.05	0.15±0.01
Tonalin®	0.24±0.04	0.23±0.06	0.12±0.02	0.13±0.03	0.17±0.04
ΣC18					
Control	46.25±1.07	44.24±0.66	45.08±0.37	45.27±0.13	44.15±0.59
Hidrolizado	53.00±1.01	52.23±9.59	51.43±0.88	52.81±0.39	52.06±2.37
Tonalin®	47.10±4.64	45.14±0.26	47.58±0.97	47.19±0.31	46.07±1.02
SFA					

Control	67.01±0.63	66.57±0.45	67.67±0.16	67.70±0.05	69.09±1.23
Hidrolizado	57.35±1.46	58.11±11.59	59.65±0.39	58.70±0.60	57.93±1.53
Tonalin®	64.02±3.55	65.25±0.32	64.57±0.44	64.88±0.06	66.00±0.76
MUFA					
Control	28.45±0.77	28.66±0.69	28.22±0.17	28.23±0.10	27.81±0.11
Hidrolizado	30.24±0.37	29.73±1.90	29.39±0.73	30.42±0.14	29.35±1.51
Tonalin®	29.61±2.72	29.83±0.02	30.38±0.40	30.15±0.14	29.22±0.59
PUFA					
Control	4.10±0.30	4.06±0.06	3.86±0.06	3.79±0.13	3.80±0.16
Hidrolizado	12.23±1.04	11.63±0.17	10.67±0.19	10.56±0.21	12.48±0.01
Tonalin®	5.37±0.79	4.35±0.33	4.23±0.04	4.31±0.04	4.20±0.15

Ver Tabla 33 para abreviaciones.

Tabla 35b. Grupos homogéneos entre medias determinados por el Test de Tukey HSD ($p<0.05$) para C18s y principales grupos de ácidos grasos (% del total de ácidos grasos) en yogures de leche de cabra en función de la fuente lipídica y el tiempo de almacenamiento en refrigeración.

Factores	**Fuente lipídica**			**Tiempo (días)**				
	Control	**Hidrolizado**	**Tonalin®**	**0**	**1**	**14**	**28**	**35**
C18:2	a	b	a	a	a	a	a	a
C18:2 *c*-9,*t*-11 CLA	a	a	b	a	a	a	a	a
C18:2 *t*-10,*c*-12 CLA	a	b	b	a	a	a	a	a
ΣC18	a	b	a	a	a	a	a	a
SFA	b	a	b	a	a	a	a	a
MUFA	a	b	b	a	a	a	a	a
PUFA	a	b	a	a	a	a	a	a

Ver Tabla 33 para abreviaciones.

L. casei ha demostrado aumentar el contenido en CLA (**Tabla 33**). Al igual que sucedía con el yogur, el contenido en ácido linoleico fue superior en los suplementados con HSO y el contenido en los isómeros estudiados de CLA fue superior en los suplementados con Tonalin® ($p<0.05$) (**Tabla 36a**). En las LF suplementadas con HSO el contenido máximo del isómero *cis*-9, *trans*-11-CLA (0.99%) se alcanzó a día 14 de almacenamiento mientras que a día 28 para el isómero *trans*-10, *cis*-12-CLA (0.17%). Mientras que el nivel del isómero *cis*-9, *trans*-11-CLA se mantuvo constante a lo largo del almacenamiento, el contenido del isómero *trans*-10, *cis*-12-CLA aumentó

gradualmente hasta el día 28 de almacenamiento tanto para los suplementados con HSO como con Tonalin® ($p<0.05$).

Tabla 36a. Contenido en C18s y principales grupos de ácidos grasos (% del total de ácidos grasos) en leche de leche de cabra fermentada con *L. casei* en función de la fuente lipídica y el tiempo de almacenamiento en refrigeración (media ± error estándar).

	Tiempo (días)				
	0	1	14	28	35
C18:2					
Control	2.79±0.06	2.83±0.26	2.75±0.05	2.70±0.02	2.69±0.09
Hidrolizado	10.49±0.58	11.14±0.42	9.23±0.23	9.81±0.36	10.81±0.03
Tonalin®	2.61±0.15	2.68±0.05	2.74±0.02	2.66±0.05	2.58±0.14
C18:2 *c*9,*t*11-CLA					
Control	0.90±0.07	0.98±0.01	0.91±0.03	0.94±0.01	0.94±0.00
Hidrolizado	0.89±0.01	0.95±0.01	0.99±0.08	0.96±0.06	0.88±0.02
Tonalin®	1.32±0.21	1.25±0.14	1.31±0.04	1.40±0.00	1.35±0.36
C18:2 *t*10,*c*12-CLA					
Control	n.d.	n.d.	n.d.	n.d.	n.d.
Hidrolizado	0.10±0.00	0.15±0.04	0.15±0.02	0.17±0.02	0.12±0.01
Tonalin®	0.16±0.02	0.17±0.00	0.23±0.01	0.28±0.04	0.27±0.03
ΣC18					
Control	43.28±0.45	44.14±0.27	45.65±0.64	45.03±0.02	44.28±0.14
Hidrolizado	51.78±0.30	53.17±0.08	52.34±0.37	52.34±0.31	50.03±0.39
Tonalin®	46.02±0.28	45.05±1.60	49.09±0.54	46.80±0.50	46.59±1.21
SFA					
Control	68.75±0.95	67.93±1.00	67.49±0.20	67.83±0.06	68.42±0.02
Hidrolizado	58.38±0.33	56.42±0.11	58.59±0.95	58.71±0.29	59.79±0.33
Tonalin®	67.46±0.47	65.99±1.09	67.07±0.33	65.16±0.96	65.74±0.89
MUFA					
Control	27.26±0.98	27.86±0.85	28.51±0.21	28.04±0.02	27.70±0.06
Hidrolizado	29.84±0.28	30.85±0.83	30.59±0.53	29.95±0.23	28.08±0.42
Tonalin®	29.96±0.52	29.55±0.77	31.21±0.47	29.74±0.62	29.04±0.30
PUFA					
Control	3.90±0.04	4.00±0.24	3.79±0.11	3.82±0.01	3.77±0.08
Hidrolizado	11.64±0.63	12.38±0.51	10.52±0.33	11.08±0.31	11.92±0.05
Tonalin®	4.22±0.15	4.21±0.35	4.47±0.06	4.53±0.06	4.92±0.55

Ver Tabla 33 para abreviaciones.

Tabla 36b. Grupos homogéneos entre medias determinados por el Test de Tukey HSD ($p<0.05$) para C18s y principales grupos de ácidos grasos (% del total de ácidos grasos) en leche de cabra fermentada con *L. casei* en función de la fuente lipídica y el tiempo de almacenamiento en refrigeración.

Factores	**Fuente lipídica**			**Tiempo (días)**				
	Control	**Hidrolizado**	**Tonalin®**	**0**	**1**	**14**	**28**	**35**
C18:2	a	b	a	ab	b	a	ab	ab
C18:2 *c*9,*t*11-CLA	a	a	b	a	a	a	a	a
C18:2 *t*10,*c*12-CLA	a	b	c	a	a	a	a	a
ΣC18	a	c	b	a	ab	b	ab	a
SFA	c	a	b	ab	a	ab	ab	b
MUFA	a	b	b	a	b	b	ab	a
PUFA	a	c	b	ab	b	a	ab	ab

Ver Tabla 33 para abreviaciones.

4.2.3.4. Evolución de los recuentos microbiológicos en las leches fermentadas durante el almacenamiento

En cuanto al **yogur**, el uso de diferentes fuentes lipídicas, el tiempo de almacenamiento y su interacción influyó de forma significativa sobre los recuentos de estreptococos y lactobacilos ($p<0.01$) (**Figura 13**). A día 28 de almacenamiento, en los yogures suplementados con HSO, los recuentos de estreptococos y lactobacilos disminuyeron significativamente. El efecto antibacteriano del ácido linoleico libre es conocido desde hace años; el efecto inhibitorio depende de la cepa bacteriana y los niveles de disponibilidad del ácido graso.

En cuanto a la LF con ***L. casei***, no se encontró interacción estadística entre la fuente lipídica y el tiempo de almacenamiento. A lo largo del almacenamiento, los recuentos medios de las LF control (8.27 ± 0.04 log UFC/g) y suplementadas con Tonalin® (8.26 ± 0.06 log UFCg) fueron superiores ($p<0.05$) a los de las LF suplementadas con HSO (8.15 ± 0.04 log UFC/g) (**Figura 14**). Mientras que los recuentos a día 1 y 14 de almacenamiento fueron similares, a día 28 y 35 disminuyeron significativamente.

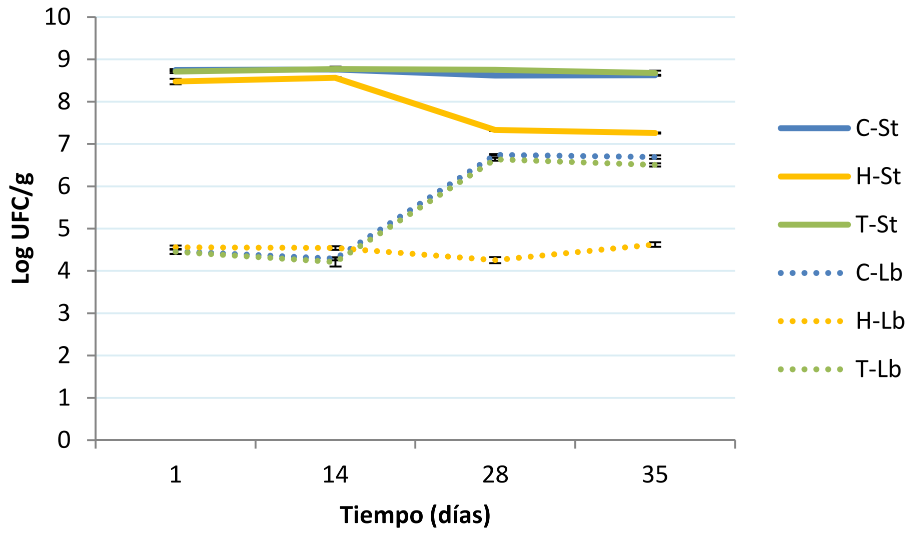

Figura 13. Efecto del tiempo de almacenamiento sobre los recuentos de estreptococos (St) y lactobacilos (Lb) en los yogures de leche de cabra con diferentes fuentes lipídicas [control (C), yogur con aceite de girasol hidrolizado (H) y yogur con Tonalin® (T)].

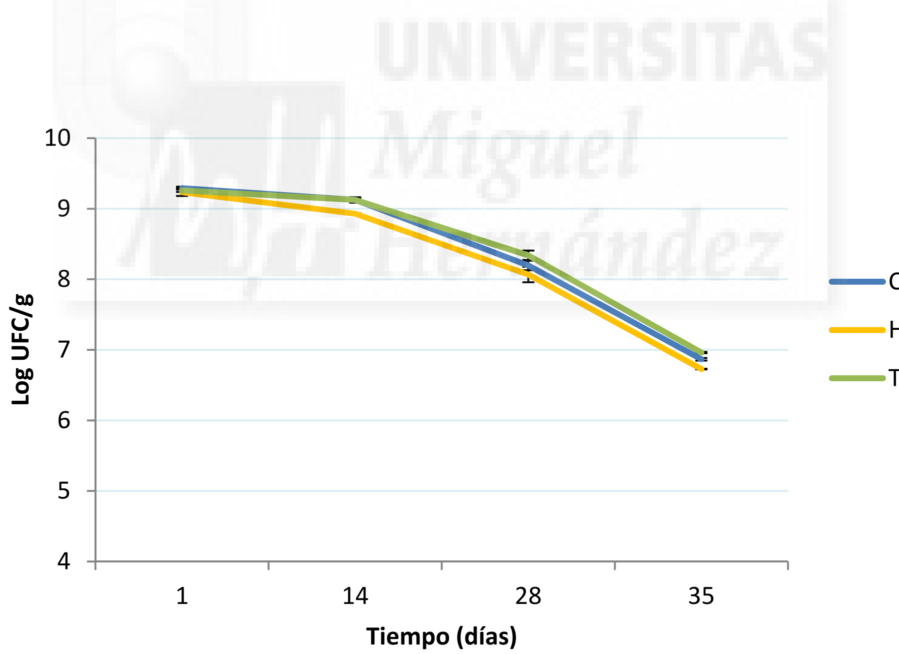

Figura 14. Efecto del tiempo de almacenamiento sobre los recuentos de *L. casei* en la leche de cabra fermentada con *L. casei* con diferentes fuentes lipídicas [control (C), yogur con aceite de girasol hidrolizado (H) y yogur con Tonalin® (T)].

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Capítulo 5: Conclusiones

En base a los resultados de la presente Tesis Doctoral se obtienen las siguientes conclusiones:

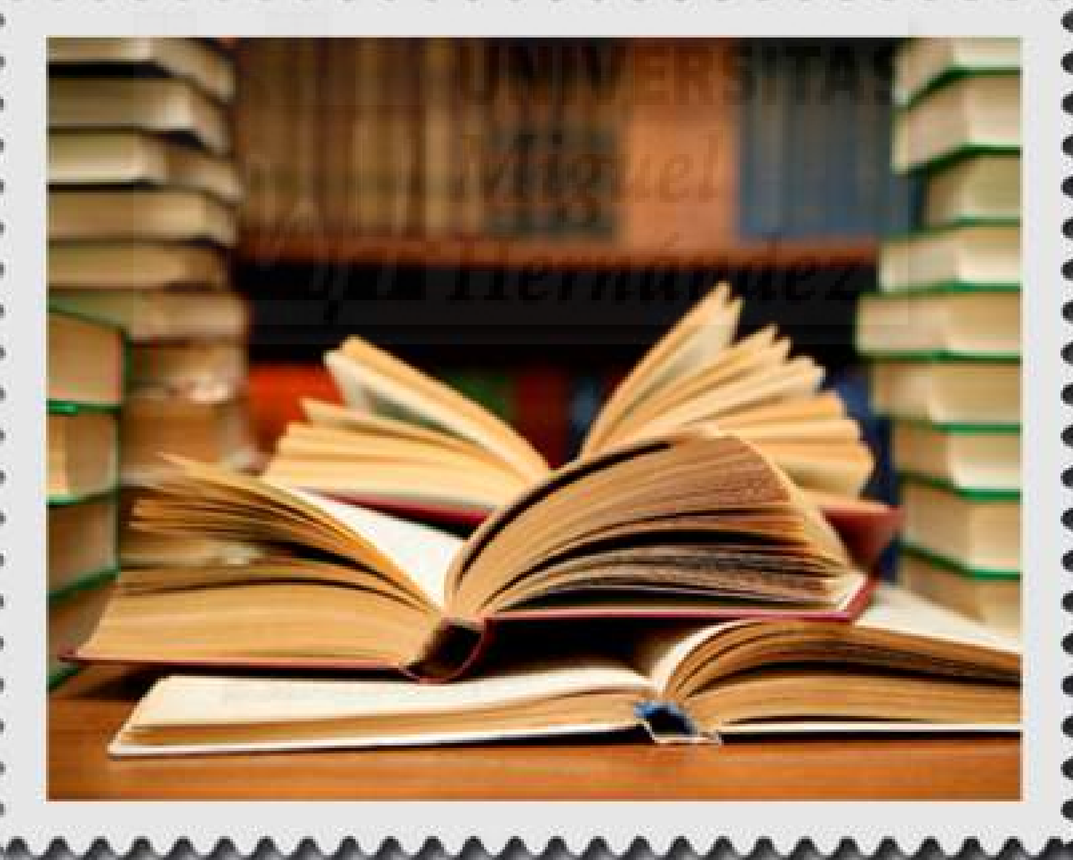
1. **Las pastas y aguas de escaldado de las industrias del dátil y membrillo pueden usarse como ingredientes en la formulación de alimentos funcionales por su contenido en fibra y antioxidantes naturales. Su uso es adecuado a diferentes matrices alimentarias debido a sus diferentes características**:
    a. La pasta y agua de escaldado del procesado de dátil Medjoul es rica en azúcares reductores y de pH cercano a la neutralidad lo que permite aplicarlo a alimentos de diversa naturaleza.
    b. Las pastas y aguas de escaldado de dátil Confitera y de membrillo presentan un elevado contenido en compuestos fenólicos y actividad antioxidante, su pH es adecuado para su incorporación a alimentos ácidos.
2. **Las aguas de escaldado de dátil pueden adicionarse en la elaboración de yogures teniendo en cuenta las siguientes particularidades** :
    a. Las aguas de escaldado provocan modificaciones del color de los yogures, y también de la textura de manera vinculada a los cambios de pH.
    b. El agua de escaldado de dátil Medjoul es apropiada para la elaboración de yogures desde un punto de vista sensorial y funcional.
    c. El agua de escaldado dátil Confitera proporciona las mejores propiedades antioxidantes, en cambio, el estado de inmadurez del fruto reduce la calificación sensorial de los yogures, lo que limita la cantidad de agua de escaldado a incorporar.
3. **El agua de escaldado de membrillo presenta efecto antimicrobiano frente a estreptococos y lactobacilos por su riqueza en fenoles y ocasiona una escasa valoración sensorial de los yogures. Su aplicación a yogur no es adecuada.**
4. **Las leches fermentadas con zumo de granada tienen una elevada capacidad antioxidante. El zumo de granada puede incorporarse hasta en un 40% en la formulación de leches fermentadas atendiendo a las siguientes particularidades**:

a. Leche y zumo deben pasteurizarse separadamente para evitar la desestabilización de las proteínas de la leche.
b. Los yogures con de zumo de granada desarrollan menor acidez, alargan el proceso de fermentación y poseen menor población de cultivos del yogur que los controles sin zumo, a causa del efecto inhibidor de los compuestos fenólicos de la granada. Estos yogures poseen un color atractivo y son positivamente calificados en aceptación sensorial.

5. **Las interacciones proteínas lácteas-polifenoles no reducen el efecto antioxidante de la leche fermentada con zumo de granada. El color y la capacidad antioxidante se mantienen estables durante el almacenamiento en refrigeración de los yogures**:
    a. Los yogures con zumo de granada poseen una elevada actividad antioxidante. Las interacciones proteínas-polifenoles dependen de la naturaleza del compuesto: el ácido elágico permanece en la fracción soluble de la leche, en cambio las punicalaginas siempre quedan retenidas en la fase proteica. Las antocianinas permanecen mayoritariamente con las proteínas: cianidin-3,5-*O*-diglucósido permanece en su totalidad en la fase proteica, mientras que el delfinidin-3,5-*O*-diglucósido apenas interacciona con ellas.
    b. Durante la fermentación y almacenamiento en refrigeración de 28 días las antocianinas individuales se degradan, las formas diglucosiladas son más estables a la fermentación que las monoglucosiladas. Los parámetros de color de los yogures no se ven afectados por esta degradación de antocianinas durante el almacenamiento.
6. **El ácido linoleico conjugado (CLA) presente de forma natural en la leche aumenta a causa del proceso de fermentación:**
    a. Este aumento es mayor cuando se incorpora a la leche, previo a la fermentación (cultivos de yogur YF-L811 Yo-Flex® y *Lactobacillus casei* LC-01 nu-trish®), ácido linoleico libre que es utilizado por las bacterias como precursor de CLA. El contenido en CLA se mantiene estable durante el almacenamiento frigorífico de ambas leches fermentadas.

b. La leche de cabra solo presenta el isómero *cis*-9*, trans*-11-CLA, con la adición de ácido linoleico a la leche las bacterias sintetizan el isómero *trans*-10, *cis*-12-CLA.
c. Las leches fermentadas comercializadas en España elaboradas con leche de vaca aportan entre 0 y 93.3 mg de CLA por ración de 100 g en función del contenido graso de la leche (0-10%).

Capítulo 6: Bibliografía



A-

Adhikari, K., Grün, I. U., Mustapha, A. y Fernando, L. N. (2002). Changes in the profile of organic acids in plain set and stirred yogurts during manufacture and refrigerated storage. *Journal of Food Quality*, 25, 435-451.

Ahmed, J., Almusallam, A. y Al-Hooti, S. N. (2013). Isolation and characterization of insoluble date (*Phoenix dactylifera* L.) fibers. *LWT-Food Science and Technology*, 50, 414-419.

Ahn, J., Grun, I. U. y Mustapha, A. (2007). Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology*, 24, 7–14.

Ajila, C. M., Aalami, M., Leelavathi, K., Rao, U. J. S. P. (2010).Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science and Emerging Technologies*, 11(1), 219-224.

Akalin, S., Siddik, G. y Gulfem, U. (2006). Functional properties of bioactive components of milk fat in metabolism. *Pakistan Journal of Nutrition*, 5, 194-197.

Albers, R., van der Wielen, R. P., Brink, E. J., Hendriks, H. F., Dorovska-Taran, V. N. y Mohede, I. C. (2003). Effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. *European Journal of Clinical Nutrition*, 57, 595–603.

Alexander, R. J. (1997). Moving toward low-calorie dairy products. *Food Product Design*, 7(1), 74-98.

Al-Farsi, M. H. y Lee, C. Y. (2008). Nutritional and functional properties of dates: a review. *Critical Reviews in Food Science and Nutrition*, 48(10), 877-887.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M. y Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. *Food Chemistry*, 104, 943-947.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M. y Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*, 53, 7592-7599.

UNIVERSITAS Miguel Hernández

Alonso, L., Cuesta, E. P. y Gilliland, E. (2003). Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. *Journal of Dairy Science*, 86, 1941-1946.

Al-Shahib, W. y Marshall, R. J. (2002). Dietary fibre content of dates from 13 varieties of date palm *Phoenix dactylifera* L. *International Journal of Food Science and Technology*, 37, 719-721.

Al-Shahib, W. y Marshall, R. J. (2003). The fruit of the date palm: Its possible use as the best food for the future?. *International Journal of Food Sciences and Nutrition*, 54(4), 247-259.

Amorós, A., Petrel, M. T., Almansa, M. S., Botella, M. A., Zapata, P. J. y Serrano, M. (2009). Antioxidant and nutritional properties of date fruit from Elche grove as affected by maturation and phenotypic variability of date palm. *Food Science and Technology International*, 15, 65-72.

Andreoli, M. F., Gonzalez, M. A., Martinelli, M. I., Mocchiutti, N. O. y Bernal, C. A. (2009). Effects of dietary conjugated linoleic acid at high-fat levels on triacylglycerol regulation in mice. *Nutrition*, 25, 445-452.

AOAC. Official methods of analysis (17th ed. Vol. AOAC), Gaithersburg, MD, 2000.

Aranceta, J. y Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: A systematic review. *British Journal of Nutrition*, 107(SUPPL. 2), S8-S22.

Ares, G., Paroli, C., y Harte, F. (2006). Measurement of firmness of stirred yogurt in routine quality control. *Journal of Food Quality*, 29, 628-642.

Aro, A., Mannisto, S., Salminen, I., Ovaskainen, M. L., Kataja, V. y Uusitupa, M. (2000). Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutrition and Cancer*, 38, 151–157.

Aslam, J., Khan, S. H. y Khan, S. A. (2013). Quantification of water soluble vitamins in six date palm (*Phoenix dactylifera* L.) cultivar’s fruits growing in Dubai, United Arab Emirates, through high performance liquid chromatography. *Journal of Saudi Chemical Society*, 17, 9-16.

Attar-Bashi, N. M., Weisinger, R. S., Begg, D. P., Li, D. y Sinclair, A. J. (2007). Failure of conjugated linoleic acid supplementation to enhance biosynthesis of docosahexaenoic acid from alpha-linolenic acid in healthy human volunteers. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 76, 121–130.

Aviram, M. y Dornfeld, L. (2001). Pomegranate juice consumption inhibits serum angiotensin-converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis*, 158(1), 195–198.

Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M., Coleman, R., Hayek, T., Presser, D. y Fuhrman, B. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apoliprotein E-deficient mice. *American Journal of Clinical Nutrition*, 71, 1062-1076.

Awaisheh, S. S., Haddadin, M. S. Y. y Robinson, R. K. (2005). Incorporation of selected nutraceuticals and probiotic bacteria into a fermented milk. *International Dairy Journal*, 15, 1184-1190.

Azain, M. J., Hausman, D. B., Sisk, M. B., Flatt, W. P. y Jewel, D. E. (2000). Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. *Journal of Nutrition*, 130, 1548-1554.

-B-

Balasundram, N., Sundram, K. y Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99, 191-203.

Baliga, M. S., Baliga, B. R. V., Kandathil, S. M., Bhat, H. P. y Vayalil, P. K. (2011). A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food Research International*, 44, 1812-1822.

Banu, J., Bhattacharya, A., Rahman, M., O'Shea, M. y Fernandes, G. (2006). Effects of conjugated linoleic acid and exercise on bone mass in young male Balb/C mice. *Lipids in Health and Disease*, 5, art. no. 7.

Baraem, I., Imad, H., Riad, B., Yehia, M. y Jeya, H. (2006). Physicochemical characteristics and total quality of five date varieties grown in the United Arab Emirates. *International Journal of Food Science and Technology,* 41,919–926.

Bassaganya-Riera, J., Hontecillas, R. y Beitz, D. C. (2002). Colonic anti-inflammatory mechanisms of conjugated linoleic acid. *Clinical Nutrition*, 21, 451–459.

Bassaganya-Riera, J., Pogranichniy, R. M., Jobgen, S. C., Halbur, P. G., Yoon, K. J., O'Shea, M., Mohede, I., Hontecillas, R. (2003). Conjugated linoleic acid ameliorates viral infectivity in a pig model of virally induced immunosuppression. *The Journal of Nutrition*, 133, 3204–3214.

Basu, A. y Penugonda, K. (2009). Pomegranate juice: a heart-healthy fruit juice. *Nutrition Reviews*, 67(1), 49–56.

Bautista, E. S., Dahiya, R. S., Speck, M. L. (1966). Identification of compounds causing symbiotic growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk. *Journal of Dairy Research*, 33, 299-307.

Beaulieu, J., Dupont, C. y Lemieux, P. (2006). Whey proteins and peptides: beneficial effects on immune health. *Therapy*, 3, 69-78.

Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A. y Del Río, J. A. (1999). Antioxidant activity of phenolics extracted from *Olea europea* L. leaves. *Food Chemistry*, 68, 457-462.

Benjamin, S. y Spener, F. (2009). Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutrition & Metabolism*, 6, art. no. 36.

Berge, G. M., Ruyter, B. y Asgard, T. (2004). Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquaculture*, 237, 365–380.

Berven, G., Bye, A., Hals, O., Blankson, H., Fagertun, H., Thom, E., Wadstein, J. y Gudmundsen, O. (2000). Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers. *European Journal of Lipid Science and Technology*, 102, 455–462.

Besbes, S., Drira, L., Blecker, C., Deroanne, C. y Attia, H. (2009). Adding value to hard date (*Phoenix dactylifera* L.): compositional, functional and sensory characteristics of date jam. *Food Chemistry*, 112, 406-411.

Betanzos-Cabrera, G., Guerrero-Solano, J. A., Martínez-Pérez, M. M., Calderón-Ramos, Z. G., Belefant, H. y Cancino-Diaz, J. C. (2011). Pomegranate juice increases levels os paraoxonase1 (PON1) expression and enzymatic activity in streptozotocin-induced diabetic mice fed with a high-fat diet. *Food Research International*, 44, 1381-1385.

Bhat, Z. F. y Bhat, H. (2011a). Functional meat products: a review. *International Journal of Meat Science*, 1(1), 1-14.

Bhat, Z. F. y Bhat., H. (2011b). Milk and dairy products as functional foods: a review. *International Journal of Dairy Sciences*, 6(1), 1-12.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J. y Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease. *Journal of Nutritional Biochemistry*, 17(12), 789–810.

Biglari, F., Alkarkhi, A. F. M., Easa, A. M. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chemistry*, 107, 1636–1641.

Bisig, W., Eberhard, P., Collomb, M. y Rehberger, B. (2007). Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products – a review. *Lait*, 87, 1-19.

Blankson, H., Stakksetad, J. A., Fagertun, H., Thom, E., Wadstein, J. y Gudmundsen, O. (2000). Conjugated linoleic acid reduces body fat mass in overweight and obese humans. Journal of Nutrition, 130, 2943-2948.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, P. M., Albertini, M. C. y Piatti, E. (2005). Raw Millefiori honey is packed full of antioxidants. *Food Chemistry*, 97, 217–222.

Bonjour, J.-P., Benoit, V., Payen, F., Kraenzlin, M. (2013). Consumption of yogurts fortified in vitamin D and calcium reduces serum parathyroid hormone and markers of bone resorption: a double-blind randomized controlled trial in institutionalized elderly women. *Journal of Clinical Endocrinology and Metabolism*, 98(7), 2915-2921.

Boyazoglu, J. y Morand-Fehr. (2001). Mediterranean dairy sheep and goat products and their quality. A critical review. *Small Ruminant Research*, 40, 1-11.

Branca, F. y Rossi, L. (2002). The role of fermented milk in complementary feeding of young children: lessons from transition countries. *European Journal of Clinical Nutrition*, 56, 16-20.

Brand-Williams, W., Cuvelier, M. E. y Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28, 25-30.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56, 317-333.

Brown, E. M. (1984). Interaction of β-lactoglobulin and α-lactalbumin with lipids: a review. *Journal of Dairy Science*, 67, 713-722.

Brown, J. M. y McIntosh, M. K. (2003). Conjugated linoleic acid in humans: regulation of adiposity and insulin sensitivity. *The Journal of Nutrition*, 133, 3041–3046.

Brownbill, R. A., Petrosian, M. y Ilich, J. Z. (2005). Association between dietary conjugated linoleic acid and bone mineral density in postmenopausal women. *Journal of the American College of Nutrition*, 24, 177–181.

-C-

Calero, J., Luna, D., Sancho, E. D., Luna, C., Bautista, F. M., Romero, A. A., Posadillo, A. y Verdugo, C. (2014). Development of a new biodiesel that integrates glycerol, by using CaO as heterogeneous catalyst, in the partial methanolysis of sunflower oil. *Fuel*, 122, 94-102.

Çam, M., Hışıl, Y. y Durmaz, G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry*, 112, 721–726.

Cardarelli, H. R., Buriti, F. C. A., Castro, I. A. y Saad, S. M. I. (2008). Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially synbiotic petit-suisse cheese. *LWT-Food Science and Technology*, 41, 1037-1046.

Carpenter, R., O'Grady, M. N., O'Callaghan, Y. C., O'Brien, N. M. y Kerry, J. P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, 76, 604–610.

Cashman, K. D. (2006). Milk minerals (including trace elements) and bone health. *International Dairy Journal*, 16, 1389-1398.

Celik, S. y Bakirci, I. (2003). Some properties of yoghurt produced by adding mulberry pekmez (concentrated juice). *International Journal of Dairy Technology*, 56(1), 26-29.

Ćetković, G., Savatović, S., Čanadanović-Brunet, J., Djilas, S., Vulić, J., Mandić, A. y Četojević-Simin, D. (2012). Valorisation of phenolic composition, antioxidant and cell growth activities of tomato waste. *Food Chemistry*, 133(3), 938-945.

Chafer, M., Ortolá, M. D., Chiralt, A. y Fito, P. (2000). Aprovechamiento de la corteza de cítricos mediante deshidratación osmótica con pulso de vacío. *Alimentación Equipos y Tecnología*, 9, 55-61.

Chajes, V., Lavillonniere, F., Maillard, V., Giraudeau, B., Jourdan, M. L., Sebedio, J. L., Bougnoux, P. (2003). Conjugated linoleic acid content in breast adipose tissue of breast cancer patients and the risk of metastasis. *Nutrition and Cancer*, 45, 17–23.

Changhua, L., Jindong, Y., Defa, L., Lidan, Z., Shiyan, Q. y Jianjun, X. (2005). Conjugated linoleic acid attenuates the production and gene expression of proinflammatory cytokines in weaned pigs challenged with lipopolysaccharide. *The Journal of Nutrition*, 135, 239–244.

Chong, M. F.-F., Macdonald, R. y Lovegrove, J. A. (2010). Fruit polyphenols and CVD risk: a review of human intervention studies. *British Journal of Nutrition*, 104, S28-S39.

Chu, Y.-F., Sun, J., Wu, X. y Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry*, 50, 6910-6916.

Clare, D. A. y Swaisgood, H. E. (2000). Bioactive milk peptides: a prospectus. *Journal of Dairy Science*, 83, 1187-1195.

Colakoglu, H. y Gursoy, O. (2011). Effect of lactic adjunct cultures on conjugated linoleic acid (CLA) concentration of yogurt drink. *Journal of Food, Agriculture & Environment*, 9, 60-64.

Collomb, M., Schmid, A., Sieber, R., Wechsler D. y Ryhänen, E. L. (2006) Conjugated linoleic acid in milk fat: variation and physiological effects. *International Dairy Journal*, 16, 1347-1361.

-D-

da Silva, L. M. R., De Figueiredo, E. A. T., Ricardo, N. M. P. S., Vieira, I. G. P., De Figueiredo, R. W., Brasil, I. M. y Gomes, C. L. (2014). Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. *Food Chemistry*, 143, 398-404.

Daniel, C. R., McCullough, M. L., Patel, R. C., Jacobs, E. J., Flanders, W. D., Thun, M. J. y Calle, E. E. (2009). Dietary intake of ω-6 and ω-3 fatty acids and risk of colorectal cancer in a prospective cohort of US men and women. *Cancer Epidemiology, Biomarkers & Prevention*, 18, 516-525.

Das, U. N. (2006). Essential fatty acids: biochemistry, physiology and pathology. *Biotechnology Journal*, 1(4), 420-439.

Dave, R. I. y Shah, N.P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, 7, 31-41.

Dave, R. I., Ramaswamy, N. y Baer, R. J. (2002). Changes in fatty acid composition during yogurt processing and their effects on yogurt and probiotic bacteria in milk procured from cows fed different diets. *Australian Journal of Dairy Technology*, 57, 197-202.

DeLany, J. P. y West, D. B. (2000). Changes in body composition with conjugated linoleic acid. *The Journal of the American College of Nutrition*, 19, 487S-493S.

Destaillats, F., Berdeaux, O., Sebedio, J. L., Juaneda, P., Gregoire, S., Chardigny, J. M., Bretillon, L. y Angers, P. (2005). Metabolites of conjugated isomers of alpha-linolenic acid (CLnA) in the rat. *Journal of Agricultural and Food Chemistry*, 53(5), 1422–1427.

Devatkal, S.K., Narsaiah, K., Borah, A. (2010). Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. *Meat Science*, 85(1), 155-159.

Dilzer, A. y Park, Y. (2012). Implication of conjugated linoleic acid (CLA) in human health. *Critical Reviews in Food Science and Nutrition*, 52(6), 488-513.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, E. B. y Roberfroid, M. B. (1999). Scientific concepts of functional foods in Europe. Consensus document. *British Journal of Nutrition*, 81, 1-27.

Djilas, S., Čanadanović-Brunet, J., Ćetković, G. (2009). By-products of fruits processing as a source of phytochemicals. *Chemical Industry and Chemical Engineering Quarterly*, 15(4), 191-202.

Domínguez-Perles, R., Martínez-Ballesta, M. C., Carvajal, M., García-Viguera, C. y Moreno, D. A. (2010). Broccoli-derived by-products - a promising source of bioactive ingredients. *Journal of Food Science*, 75(4), C383-C392.

Donovan, S. M. (2006). Role of human milk components in gastrointestinal development: current knowledge and future needs. *Journal of Pediatrics*, 149, S49-S61.

Doughty, E. (1995). Separation of acids, carbohydrates and fermentation products by HPLC. *Lebensmittel and Biotechnologie*, 12(3), 100-101.

Doyle, L., Jewell, C., Mullen, A., Nugent, A. P., Roche, H. M. y Cashman, K. D. (2005). Effect of dietary supplementation with conjugated linoleic acid on markers of calcium and bone metabolism in healthy adult men. *European Journal of Clinical Nutrition*, 59, 432–440.

Durán, E. (2013). Alimentación y bebidas: innovando con el viento en cara. *Alimarket*, Febrero 2013. Edición on-line, sección Alimentación, 11 pp.

-E-

EFSA (2012). European Food Safety Association, scientific opinion on the substantiation of a health claim related to polyphenols in olive and maintenance of normal blood HDL cholesterol concentrations (ID 1639, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *The EFSA Journal*, 10(8): 2848 [9 pp.]

EFSA (2009). European Food Safety Association, scientific opinion: plant stanols and plant sterols and blood LDL-cholesterol. *The EFSA Journal*, 1175, 1-9.

Elfalleh, W., Tlili, N., Nasri, N., Yahia, Y., Hannachi, H., Chaira, N., Ying, M. y Ferchichi, A. (2011). Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits. *Journal of Food Science*, 76 (5), C707-C713.

Elluch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. y Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review. *Food Chemistry*, 124, 411-421.

El-Zoghbi, M. (1994). Biochemical changes in some tropical fruits during ripening. *Food Chemistry*, 49, 33-37.

Espírito Santo, A. P., Perego, P., Converti, A. y Oliveira, M. N. (2011). Influence of food matrices on probiotic viability - a review focusing on the fruity bases. *Trends in Food Science & Technology*, 22, 377-385.

Eurostat (2010). Eurostat: Causes of Death. http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/. Última consulta: 16/12/2013.

-F-

Falsaperla, M., Morgia, G., Tartarone, A., Ardito, R. y Romano, G. (2005). Support ellagic acid therapy in patients with hormone refractory prostate cancer (HRPC) on standard chemotherapy using vinorelbine and estramustine phosphate. *European Urology*, 47(4), 449-454.

FAO (Food and Drug Organization) (2014). Statistical databases. http://faostat.fao.org. Última consulta: 3/2/2014.

Faria, A. y Calhau, C. (2011). The bioactivity of pomegranate: impact on health and disease. *Critical Reviews in Food Science and Nutrition*, 51, 626-634.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C. I, Angioni, A., Dessi, S., Marzouki, N. y Cabras, P. (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *Journal of Agricultural and Food Chemistry*, 55 (3), 963-969.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessi, S., Marzouki, N. y Cabras, P. (2008). Comparative analysis of polyphenols profiles and antioxidant and antimicrobial activities of Tunisian pome fruit pulp and peel aqueous acetone extracts. *Journal of Agricultural and Food Chemistry*, 56 (3), 1084-1090.

FDA (U. S. Food and Drug Administration). (2013). Code of Federal Regulations, Title 21, Volume 2, URL http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.83. Consultado 23/01/2014

Fernández-López, J., Fernández-Ginés, J. M., Alesón-Carbonell, L., Sendra, E., Sayas-Barberá, E. y Pérez-Álvarez, J. A. (2004). Application of functional citrus by-products to meat products. *Trends in Food Science & Technology*, 15, 176–185.

Fischer, U. A., Dettmann, J. S., Carle, R. y Kammerer, D. R. (2011). Impact of processing and storage on the phenolic profiles and contents of pomegrante (*Punica granatum* L.) juices. *European Food Research and Technology*, 233, 797-816.

Fischer, U. A., Jaksch, A. V., Carle, R. y Kammerer, D. R. (2013). Influence of origin source, different fruit tissue and juice extraction methods on anthocyanin, phenolic acid, hydrolysable tannin and isolariciresinol contents of pomegranate (*Punica granatum* L.) fruits and juices. *European Food Research and Technology*, 237, 209-221.

Flachs, P., Rossmeisl, M., Bryhn, M. y Kopecky, J. (2009). Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clinical Science*, 116(1), 1-16.

Förstermann, U. (2008). Oxidative stress in vascular disease: causes, defense, mechanisms and potential therapies. *Nature Clinical Practice Cardiovascular Medicine*, 5, 338-349.

Fritsche, J. y Steinhart, H. (1998). Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. *Zeitschrift fur Lebensmittel-Untersuchung und –Forschung*, 206, 77-82.

Fuhrman, B. y Aviram, M. (2007). Pomegranate and cardiovascular diseases: pomegranate juice polyphenolic antioxidants protect against oxidative stress and atherosclerosis development. *Acta Horticulturae*, 744, 205-216.

-G-

Gambaro, A., Gimenez, A. y Burgueño, J. (2001). Sensory and instrumental evaluation of strawberry yogurt color. *Journal of Sensory Studies*, 16, 11-22.

García-Pérez, F. J., Lario, Y., Fernández-López, J., Sayas, E., Pérez-Álvarez, J. A., y Sendra, E. (2005). Effect of orange fiber addition on yogurt color during fermentation and cold storage. *Color Research and Application*, 30(6), 457-463.

Gardner, P. T., White, T. A. C., McPhail, D. B. y Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, 68, 471-474.

Gaucheron, F. (2000). Iron fortification in dairy industry. *Trends in Food Science & Technology*, 11, 403-409.

Gaullier, J. M., Halse, J., Hoye, K., Kristiansen, K., Fagertun, H., Vik, H. y Gudmundsen, O. (2005). Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. *The Journal of Nutrition*, 135, 778–784.

Gaullier, J. M., Halse, J., Hoye, K., Kristiansen, K., Fagertun, H., Vik, H. y Gudmundsen, O. (2004). Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *The American Journal of Clinical Nutrition*, 79, 1118–1125.

Gebauer, S. K., Chardigny, J.-M., Jakobsen, M. U., Lamarche, B., Lock, A. L., Proctor, S. P., Baer, D. J. (2011). Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies. *Advances in Nutrition*, 2(4), 332-354.

Genestra, M. (2007). Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cellular Signalling*, 19, 1807-1819.

George, B., Kaur, C., Khurdiya, D. S. y Kapoor, H. C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry*, 84, 45-51.

Gerhauser, C. (2008). Cancer chemopreventive potential of apples, apple juice, and apple components. *Planta Medica*, 74, 1608-1624.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M. y Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, 48, 4581-4589.

Giusti, M. M. y Wrolstad, R. E. (1996). Radish anthocyanin extract as a natural red colorant for maraschino cherries. *Journal of Food Science*, 61(4), 688-694.

Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A. y Di Cagno, R. (2002). Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. *Critical Reviews in Food Science and Nutrition*, 42, 223-239.

Gorinstein, S., Martin-Belloso, O., Park, Y.-S., Haruenkit, R., Lojek, A., Ĉíž, M. Caspi, A., Libman, I. y Trakhtenberg, S. (2001). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74, 309-315.

Gorinstein, S., Zemser, M., Haruenkit, R., Chuthakorn, R., Grauer, F., Martin-Belloso, O. y Trakhtenberg, S. (1999). Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *Journal of Nutritional Biochemistry*, 10, 367-371.

Gorissen, L., Raes, K., De Smet, S., De Vuyst, L. y Leroy, F. (2012). Microbial production of conjugated linoleic and linolenic acids in fermented foods: technological bottlenecks. *European Journal of Lipid Science and Technology*, 114, 486-491.

-H-

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartfield, P. W. y Riechel, T. L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, 46, 1887-1892.

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M. y Törrönen, A. R. (2000). Influence of domestic processing and storage on flavonol contents in berries. *Journal of Agricultural and Food Chemistry*, 48, 2960-2965.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research*, 9, 1-32.

Hamauzu, Y., Inno, T., Kume, C., Irie, M. y Hiramatsu, K. (2006). Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *Journal of Agricultural and Food Chemistry*, 54(3), 765-772.

Hamauzu, Y., Irie, M., Kondo, M. y Fujita, T. (2008). Antiulcerative properties of crude polyphenols and juice of apple, and Chinese quince extracts. *Food Chemistry*, 108(2), 488-495.

Hansel, B., Nicole, C., Lalanne, F., Tondu, F., Lassel, T., Donazzolo, Y., Ferrières, J., Krempf, M., Schlienger, J. L., Verges, B., Chapman, M. J. y Bruckert, E. (2007). Effect of low fat fermented milk enriched with plant sterols on serum lipid profile and antioxidative stress in moderate hypercholesterolemia. *American Journal of Clinical Nutrition*, 86, 790-796.

Hao, M. y Beta, T. (2012). Qualitative and quantitative analysis of the major phenolic compounds as antioxidants in barley and flaxseed hulls using HPLC/MS/MS. *Journal of the Science of Food and Agriculture*, 92 (10), 2062-2068.

Hartmann, R., Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. *Current Opinion in Biotechnology*, 18(2), 163-169.

Hashim, I. B., Khalil, A. H. y Afifi, H. S. (2009). Quality characteristics and consumer acceptance of yogurt fortified with date fibre. *Journal of Dairy Science*, 92(11), 5403-5407.

Hasnaoui, N., Mars, M., Ghaffari, S., Trifi, M., Melgarejo, P. y Hernandez, F. (2011). Seed and juice characterization of pomegranate fruits grown in Tunisia: comparison between sour and sweet cultivars revealed interesting properties for prospective industrial applications. *Industrial Crops and Products*, 33, 374-381.

Hassoun, E. A., Vodhanel, J. y Abushaban, A. (2004). The modulatory effects of ellagic acid and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of rats after subchronic exposure. *Journal of Biochemical and Molecular Toxicology*, 18(4), 196-203.

Heim, K. E., Tagliaferro, A. R. y Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, 13, 572-584.

Heinemann, T., Kullak-Ublick, G. A., Pietruck, B., y von Bergmann, K. (1991). Mechanisms of action of plant sterols on inhibition of cholesterol absorption. *European Journal of Clinical Pharmacology*, 40, 859-863.

Hernández-Ledesma, B., Amigo, L., Recio, I. y Bartolome, B. (2007). ACE-inhibitory and radical-scavenging activity of peptides derived from β-lactoglobulin f(19-25). Interactions with ascorbic acid. *Journal of Agricultural and Food Chemistry*, 55, 3392-3397.

Herrera, J. A., Shahabuddin, A. K., Ersheng, G., Wei, Y., Garcia, R. G. y Lopez-Jaramillo, P. (2005). Calcium plus linoleic acid therapy for pregnancy-induced hypertension. International Journal of Gynaecology and Obstetrics, 91(3), 221–227.

Herrman, K. M. (1995). The shikimate pathway: early steps in the biosynthesis of aromatic compounds. *Plant Cell*, 7, 907-919.

Huang, D., Ou, B. y Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53 (6), 1841-1886.

Hudson, E. (2013). Webminar: http://www.dairyreporter.com/Internet-Live-Events/Healthy-Functional-Dairy-2013, by William Reed Business Media, realizado en Diciembre de 2013.

-I-

Igarashi, M. y Miyazawa, T. (2005). Preparation and fractionation of conjugated trienes from alpha-linolenic acid and their growth-inhibitory effects on human tumor cells and fibroblasts. *Lipids*, 40(1), 109–113.

IDF (International Dairy Federation). (2013). World Dairy Situation, www.fil-idf.org/Public/Download.php?file=16550, descargado en Diciembre 2013.

Ip, C., Thompson, H. J. y Scimeca, J. A. (1994). Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Research*, 54, 1212-1215.

Iwata, T., Kamegai, T., Yamauchi-Sato, Y., Ogawa, A., Kasai, M., Aoyama, T. y Kondo, K. (2007). Safety of dietary conjugated linoleic acid (CLA) in a 12-weeks trial in healthy overweight Japanese male volunteers. *Journal of Oleo Science*, 56, 517–525.

-J-

Jahreis, G., Kraft, J., Tischendorf, F., Schöne, F. y von Loeffelholz, V. (2000). Conjugated linoleic acids: physiological effects in animal and man with special regard to body composition. *European Journal of Lipid Science and Technology*, 102, 695-7103.

Jaziri, M., Migliore-Samour, D., Casabianca-Pignede, M. R., Keddad, K., Morgat, J. L. y Jolles, P. (1992). Specific binding sites on human phagocytic blood cells for Gly-Leu-Phe and Val-Glu-Pro-Ile-Pro-Tyr, immunostimulating peptides from human milk proteins. *Biochimica et Biophysica Acta*, 1160, 251-261.

Jensen, H. D., Krogfelt, K. A., Cornett, C., Hansen, S. H. y Christensen, S. B. (2002). Hydrophilic carboxylic acids and iridoid glycosides in the juice of American and European cranberries (*Vaccinum macrocarpon* and *V. oxycoccus)*, lingonberries (*V. vitis-idaea*), and blueberries (*V. myrtillus*). *Journal of Agricultural and Food Chemistry*, 50, 6871-6874.

Jewell, C. y Cashman, K. D. (2003). The effect of conjugated linoleic acid and médium chain fatty acids on transepithelial calcium transport in human intestinal-like Caco-2 cells. *The British Journal of Nutrition*, 89, 639–647.

Jewell, C., Cusack, S. y Cashman, K. D. (2005). The effect of conjugated linoleic acid on transepithelial calcium transport and mediators of paracellular permeability in human intestinal-like Caco-2 cells. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 72, 163–171.

Jiang, J., Björck, L. y Fondén, R. (1998). Production of conjugated linoleic acid by dairy starter cultures. *Journal of Applied Microbiology*, 85, 95-102.

Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based functional foods. *Technological options for replacement of meat fats by non-meat fats. Trends in Food Science and Technology*, 18, 567-578.

Johanningsmeier, S. D. y Harris, G. K. (2011). Pomegranate as a functional food and nutraceutical source. *Annual Review of Food Science and Technology*, 2, 181-201.

Jones, P. J., Ntanios, F. Y., Raeini-Sarjaz, M. y Vanstone, C. A. (1999). Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prodent diet in hyperlipidemic men. *American Journal of Clinical Nutrition*, 69, 1144-1150.

Junta de Andalucía. (2013). Membrillo y Cítricos. http://www.juntadeandalucia.es/agriculturaypesca/portal/export/sites/default/comun/galerias/galeriaDescargas/cap/produccion-ecologica/estudios/el_membrillero_citricos.pdf, consultado en Diciembre de 2013.

Jurenka, J. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): a review. *Alternative Medicine Review*, 13(2), 128-144.

-K-

Kailasapathy, K., Harmstorf, I. y Phillips, M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. *LWT - Food Science and Technology*, 41, 1317–1322.

Karaaslan, M., Ozden, M., Vardin, H., Turkoglu, H. (2011). Phenolic fortification of yogurt using grape and callus extracts. *LWT - Food Science and Technology*, 44, 1065-1072.

Karakaya, S., El., S. N. y Taş, A. A. (2001). Antioxidant activity of some foods containing phenolic compounds. *International Journal of Food Sciences and Nutrition*, 52, 501-508.

Karar, M. G. E., Pletzer, D., Jaiswal, R., Weingart, H. y Kuhnert, N. (2013). Identification, characterization, isolation and activity against *Escherichia coli* of quince (*Cydonia oblonga*) fruit polyphenols. *Food Research International*, http://dx.doi.org/10.1016/j.foodres.2013.10.040.

Karre, L., Lopez, K. y Getty, K. J. K. (2013). Natural antioxidants in meat and poultry products. *Meat Science*, 94, 220-227.

Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K. y Kobori, M. (2003). Induction of apoptosis in cáncer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *Journal of Agricultural and Food Chemistry*, 51, 68-75.

Kaur, C. y Kapoor, H. C. (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*, 37, 153-161.

Kee, J.-I., Ganesan, P., Kwak, H.-S. (2010). Bioactive conjugated linoleic acid (CLA) in milk. *Korean Journal for Food Science of Animal Resources*, 30(6), 879-885.

Kelley, N. S., Hubbard, N. E. y Erickson, K. L. (2007). Conjugated linoleic acid isomers and cancer. *The Journal of Nutrition*, 137, 2599–2607.

Kelly, O., Cusack, S., Jewell, C. y Cashman, K. D. (2003). The effect of polyunsaturated fatty acids, including conjugated linoleic acid, on calcium absorption and bone metabolism and composition in young growing rats. *The British Journal of Nutrition*, 90, 743–750.

Khoubnasabjafari, M. y Jouyban, A. (2011). A review of phytochemistry and bioactivity of quince (*Cydonia oblonga* Mill.). *Journal of Medicinal Plants Research*, 5(16), 3577-3594.

Khurana, H. K. y Kanawjia, S. K. (2007). Recent trends in development of fermented milks. *Current Nutrition & Food Science*, 3, 91-108.

Kieliszek, M., Błazejak, S. (2013). Selenium: significance and Outlook for supplementation. *Nutrition*, 29 (5), 713-718.

Kim, D.-O., Jeong, S. W. y Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, 81, 321-326.

Klimczak, I., Małecka, M., Szlachta, M. y Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis*, 20, 313-322.

Kolanowski, W., Swiderski, F. y Berger, S. (1999). Possibilities os fish oil application for foods products enrichment with omega-3 PUFA. *International Journal of Food Science and Nutrition*, 50, 39-49.

Korhonen, H. y Pihlanto, A. (2006). Bioactive peptides: production and functionality. *International Dairy Journal*, 16, 945-960.

Kreider, R. B., Ferreira, M. P., Greenwood, M., Wilson, M. y Almada, A. L. (2002). Effects of conjugated linoleic acid supplementation during resistance training on body composition, bone density, strength, and selected hematological markers. *Journal of Strength and Conditioning Research*, 16, 325–334.

Kritchevsky, D. (2000). Antimutagenic and some other effects of conjugated linoleic acid. *The British Journal of Nutrition*, 83, 459–465.

Kritchevsky, D., Tepper, S. A., Wright, S., Czarnecki, S. K., Wilson, T. A. y Nicolosi, R. J. (2004). Conjugated linoleic acid isomer effects in atherosclerosis: growth and regression of lesions. *Lipids*, 39, 611–616.

-L-

Landete, J. M. (2012). Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. *Critical Reviews in Food Science and Nutrition*, 52(10), 936-948.

Landete, J. M. (2013). Dietary intake of natural antioxidants: vitamins and polyphenols. *Critical Reviews in Food Science and Nutrition*, 53(7), 706-721.

Larrauri, J. A. (1995). Fibra de subproductos tropicales. *Alimentación, equipos y tecnologías*, Junio, 107-111.

Larsen, T. M., Toubro, S. y Astrup, A. (2003). Efficacy and safety of dietary supplements containing conjugated linoleic acid (CLA) for the treatment of obesity-evidence from animal and human studies. *Journal of Lipid Research*, 44(12), 2234-2241.

UNIVERSITAS Miguel Hernández

Larsson, S. C., Bergkvist, L. y Wolk, A. (2005). High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. *The American Journal of Clinical Nutrition*, 82, 894–900.

Leatherhead Food International. (2006). Health-related and functional foods show year-on-year growth. *Food News*, 40, 2.

Lee, C. H., Reed, J. D. y Richards, M. P. (2006). Ability of various polyphenolic clases from cranberry to inhibit lipid oxidation in mechanically separated turkey and cooked ground pork. *Journal of Muscle Foods*, 17, 248–266.

Lee, J. H., O'Keefe, J. H., Lavie, C. J., Marchioli, R. y Harris, W. S. (2008). Omega-3 fatty acids for cardioprotection. *Mayo Clinic Proceedings*, 83, 324-332.

Lee, K. N., Pariza, M. W. y Ntambi, J. M. (1998). Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. *Biochemical and Biophysical Research Communications*, 248, 817–821.

Lee, K. W., Lee, H. J., Cho, H. Y. y Kim, Y. J. (2005). Role of the conjugated linoleic acid in the prevention of cancer. *Critical Reviews in Food Science and Nutrition*, 45, 135–144.

Levin, I., Gilboa, N., Yeselson, E., Shen, S. y Schaffer, A. A. (2000). *Fgr*, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. *Theoretical and Applied Genetics*, 100, 256–262.

Lewandowska, U., Szewczyk, K., Hrabec, E., Janecka, A. y Gorlach, S. (2013). Overview of metabolism and bioavailability enhancement of polyphenols. *Journal of Agricultural and Food Chemistry*, 61, 12183-12199.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J. y Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry, 96,* 254–260.

Lin, H., Boylston, T. D., Chang, M. J., Luedecke, L. O. y Shultz, T. D. (1995). Survey of the conjugated linoleic acid contents of dairy products. *Journal of Dairy Science*, 78, 2358-2365.

Lin, J.-K., Lin, C.-H., Liang, Y.-C., Lin-Shiau, S.-Y. y Juan, I-M. (1998). Survey of catechins, gallic acid, and methylxantines in green, oolong, pu-erh and black teas. *Journal of Agricultural and Food Chemistry*, 46, 3635-3642.

Lin, T. Y., Lin, C.-W. y Lee, C.-H. (1999). Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. *Food Chemistry*, 67, 1-5.

Lock, A. L. y Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids*, 39, 1197–1206.

Lubbers, S., Decourcelle, N., Vallet, N. y Guichard, E. (2004). Flavor release and rheology behaviour of strawberry fat-free stirred yogurt during storage. *Journal of Agricultural and Food Chemistry*, 52(10), 3077-3082.

Luna, C., Sancho, E., Luna, D., Caballero, V., Calero, J., Posadillo, A., Verdugo, C., Bautista, F. M., y Romero, A. A. (2013). Biofuel that keeps glycerol as monoglyceride by 1,3-selective ethanolysis with pig pancreatic lipase covalently immobilized on $AlPO_4$ support. *Energies*, 6, 3879-3900.

Luongo, D., Bergamo, P. y Rossi, M. (2003). Effects of conjugated linoleic acid on growth and cytokine expression in Jurkat T cells. *Immunology Letters*, 90, 195–201.

Luximon-Ramma, A., Bahorun, T. y Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritanian exotic fruits. *Journal of the Science of Food and Agriculture*, 83, 496-502.

-M-

Mahdavi, R., Nikniaz, Z., Rafraf, M. y Jouyban, A. (2010). Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. *Pakistan Journal of Nutrition*, 9, 968-972.

Manach, C., Williamson, G., Morand, C., Scalbert, A. y Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, 81, 230-242.

Martín, M. A. (1995). Estudio del contenido en fibra alimentaria de subproductos de las industrias cítricas y cerveceras. *Alimentaria*, Abril: 61-64.

Martín-Diana, A. B., Janer, C., Peláez, C. y Requena, T. (2004). Effect of milk fat replacement by polyunsaturated fatty acids on the microbiological, rheological and sensorial properties of fermented milks. *Journal of the Science of Food and Agriculture*, 84, 1599-1605.

Martínez-Navarrete, N., Camacho, M. M., Martínez-Lahuerta, J., Martínez-Monzó, J. y Fito, P. (2002). Iron deficiency and iron fortified foods – a review. *Food Research International*, 35, 225-231.

Martín-Sánchez, A. M., Cherif, S., Vilella-Esplá, J., Ben-Abda, J., Kuri, V., Pérez-Álvarez, J. A., Sayas-barberá, E. (2014). Characterization of novel intermediate food products from Spanish date palm (*Phoenix dactylifera* L., cv. Confitera) co-products for industrial use. *Food Chemistry*, 154, 269-275.

Martín-Sánchez, A. M., Ciro-Gómez, G., Sayas, E., Vilella-Esplá, J., Ben-Abda, J., Pérez-Álvarez, J. A. (2013). Date palm by-products as a new ingredient for the meat industry: application to pork liver pâté. *Meat Science*, 93, 880-887.

McCann, S. E., Ip, C., Ip, M. M., McGuire, M. K., Muti, P., Edge, S. B., Trevisan, M. y Freudenheim, J. L. (2004). Dietary intake of conjugated linoleic acids and risk of premenopausal and postmenopausal breast cancer, Western New York Exposures and Breast Cancer Study (WEB Study). *Cancer Epidemiology, Biomarkers & Prevention*, 13(9), 1480–1484.

McCrorie, T. A., Keaveney, E. M., Wallace, J. M., Binns, N. y Livingstone, M. B. E. (2011). Human health effects of conjugated linoleic acid from milk and supplements. *Nutrition Research Reviews*, 24, 206–227.

McLeod, R. S., LeBlanc, A. M., Langille, M. A., Mitchell, P. L. y Currie, D. L. (2004). Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. *The American Journal of Clinical Nutrition*, 79, 1169S–1174S.

Meadus, W. J., MacInnis, R. y Dugan, M. E. (2002). Prolonged dietary treatment with conjugated linoleic acid stimulates porcine muscle peroxisome proliferator activated receptor gamma and glutamine-fructose aminotransferase gene expression *in vivo*. *Journal of Molecular Endocrinology*, 28, 79-86.

Medjakovic, S. y Jungbauer, A. (2013). Pomegranate: a fruit that ameliorates metabolic syndrome. *Food Func*, 4, 19–39.

Melgarejo, P., Salazar, D. M. y Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology*, 211, 185-190.

Mena, P., Ascacio-Valdés, J. A., Gironés-Vilaplana, A., Del Rio, D., Moreno, D. A. y García-Viguera, C. (2014). Assessment of pomegranate wine lees as a valuable source for the recovery of (poly)phenolic compounds. *Food Chemistry*, 145, 327-334.

Mena, P., Gironés-Vilaplana, A., Martí, N. y García-Viguera, C. (2012). Pomegranate varietal wines: phytochemical composition and quality parameters. *Food Chemistry*, 133, 108-115.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D. y Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture*, 91, 1893-1906.

Meraz-Torres, L. S. y Hernández-Sánchez, H. (2012). Conjugated linoleic acid in dairy products: a review. *American Journal of Food Technology*, 7(4), 176-179.

Mezzaroba, L. F. H., Carvalho, J. E., Ponezi, A. N., Antonio, M. A., Monteiro, K. M., Possenti, A. y Sgarbieri, V. C. (2006). Antiulcerative properties of bovine α-lactalbumin. *International Dairy Journal*, 16, 1005-1112.

Mildner-Szkudlarz, S., Bajerska, J., Zawirska-Wojtasiak, R., Górecka, D. (2013). White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *Journal of the Science of Food and Agriculture*, 93(2), 389-395.

Milner, J. A. y Alison, R. G. (1999). The role of fat in child nutrition and development: summary of an ASNS work-shop. *Journal of Nutrition*, 129, 2094-2105.

Misra, A. y Khurana, L. (2008). Obesity and the metabolic syndrome in developing countries. *Journal of Clinical Endocrinology and Metabolism*, 93(11 SUPPL. 1), s9-s30.

Mohdaly, A. A. A., Hassanien, M. F. R., Mahmoud, A., Sarhan, M. A. y Smetanska, I. (2013). Phenolics extracted from potato, sugar beet, and sesame processing by-products. *International Journal of Food Properties*, 16(5), 1148-1168.

Moon, H.-S., Lee, H.-G., Chung, C.-S., Choi, Y.-J. y Cho, C.-S. (2008). Physico-chemical modifications of conjugated linoleic acid for ruminal protection and oxidative stability. *Nutrition and Metabolism*, 5, 5-16.

Moreno, O. (2012). Sector lácteo: el eterno retorno. *Alimarket*, Diciembre 2012. Edición on-line, sección Alimentación, 10 pp.

Mortazavi, S., Arzani, K. y Barzegar, M. (2010). Analysis of sugars and organic acids contents of date palm (*Phoenix dactylifera* L.) 'Barhee' during fruit development. En: IV International date palm conference 882 (pp. 793–801).

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B. y Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits *Vaccinium, Rubus* and *Ribes*. *Journal of Agricultural and Food Chemistry*, 50, 519-525.

-N-

Nagao, K. y Yanagita, T. (2005). Conjugated fatty acids in food and their health benefits. *Journal of Bioscience and Bioengineering*, 100(2), 152-157.

Nazzaro, M., Mottola, M. V., La Cara, F., Del Monaco, G., Aquino, R. P. y Volpe, M. G. (2012). Extraction and characterization of biomolecules from agricultural wastes. *Chemical Engineering Transactions*, 27, 331-336.

Nicolosi, R. J., Rogers, E. J., Kritchevsky, D., Scimeca, J. A. y Huth, P. J. (1997). Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic aterosclerosis in hypercholesterolemic hamsters. *Artery*, 22, 266–277.

-O-

O'Brien, J. (1999). Sugar profiles of cultured dairy products in the UK. *Journal of Human Nutrition and Dietetics*, 12, 245-250.

O'Connell, J. E. y Fox, P. F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *International Dairy Journal*, 11, 103-120.

OECD (2011). Organization for Economic Co-Operation and Development. Statistics Data BaseHealth Status. http://stats.oecd.org/index.aspx?DataSetCode=HEALTH_STAT#. Última consulta: 9/12/2013.

OECD (2012). Obesity and the Economics of Prevention: Fit not Fat - Spain Key Facts. http://www.oecd.org/health/health-

systems/obesityandtheeconomicsofpreventionfitnotfat-spainkeyfacts.htm. Última consulta: 26/11/2013.

Ogawa, J., Kishino, S., Ando, A., Sugimoto, S., Mihara, K. y Shimizu, S. (2005). Production of conjugated fatty acids by lactic acid bacteria. *Journal of Bioscience and Bioengineering*, 100(4), 355-364.

Onakpoya, I. J., Posadzki, P. P., Watson, L. K., Davies, L. A. y Ernst, E. (2012). The efficacy of long-term conjugated linoleic acid (CLA) supplementation on body composition in overweight and obese individuals: a systematic review and meta-analysis of randomized clinical trials. *European Journal Nutrition*, 51, 127–134.

Ong, L. y Shah, N. P. (2009). Probiotic cheddar cheese: influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. *LWT-Food Science and Technology*, 42, 1260-1268.

Ottaway, B. (2009). Fortification of beverages with vitamins and minerals. En: *Functional and Speciality Beverage Technology*, pp 71-91. Paquin, P., ed. Cambridge: Wood-head Publishing Limited.

Oukhatar, N., Peres, J. M., Bouhallab, S., Neuville, D., Bureau, F., Bouvard, G., Arhan, P. y Bougle, D. (2002). Bioavailability of caseinphosphopeptide-bound iron. *Journal of Laboratory and Clinical Medicine*, 140, 290-294.

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307-315.

Ozen, A. E., Pons, A. y Tur, J. A. (2012). Worldwide consumption of functional foods: a systematic review. *Nutrition Reviews*, 70(8), 472-481.

Özer, B. H. y Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology*, 63(1), 1-15.

Ozgen, M., Durgaç, C., Serçe, S. y Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry*, 111, 703-706.

Öztürk, B. A. y Öner, M. D. (1999). Production and evaluation of yogurt with concentrated grape juice. *Journal of Food Science*, 64(3), 530-532.

-P-

Palmquist, D. L., Lock, A.L., Shingfield, K. J. y Bauman, D. E. (2005). Biosynthesis of conjugated linoleic acid in ruminants and humans. *Advances in Food and Nutrition Research*, 50, 179–217.

Pandey, K. B. y Rizvi, S. I. (2009). Current understanding of dietary polyphenols and their role in health and disease. *Current Nutrition & Food Science*, 5, 249-263.

Papastoyiannidis, G., Polychroniadou, A., Michaelidou, A.-M., Alichanidis, E. (2006). Fermented milks fortified with B-group vitamins: vitamin stability and effect on resulting products. *Food Science and Technology International*, 12 (6), 521-529.

Park, P. W. y Goins, R. E. (1994). *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, 59, 1262-1266.

Park, Y. y Pariza, M. W. (2007). Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International*, 40, 311–323.

Park, Y., Pariza, M. W. y Park, Y. (2008). Co-supplementation of dietary calcium and conjugated linoleic acid (CLA) improves bone mass in mice. *Journal of Food Science*, 73, C556–C560.

Park, Y., Storkson, J. M., Albright, K. J., Liu, W. y Pariza, M. W. (1999). Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids*, 34, 235–241.

Parodi, P. W. (1999). Symposium: a bold new look at milk fat. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *Journal of Dairy Science*, 82, 1339-1349.

Pellegrini, A. (2003). Antimicrobial peptides from food proteins. *Current Pharmaceutical Design*, 9, 1225-1238.

Peng, Y., Horne, D. S. y Lucey, J. A. (2009). Impact of preacidification of milk and fermentation time on the properties of yogurt. *Journal of Dairy Science*, 92, 2977-2990.

Phadungath, C. (2005). The mechanism and properties of acid-coagulated milk gels. *Songklanakarin Journal of Science and Technology*, 27(2), 433-448.

Picó, C., Oliver, P., Priego, T., Sánchez, J. y Palou, A. (2006). Alimentos funcionales y obesidad: estrategias, eficacia y seguridad. *Revista Española Obesidad*, 4(3), 156-174.

Playne, M. J., Bennett, L. E. y Smithers, G. W. (2003). Functional dairy foods and ingredients. *The Australian Journal of Dairy Technology*, 58(3), 22-44.

Plourde, M., Sergiel, J. P., Chardigny, J. M., Gregoire, S., Angers, P. y Sebedio, J. L. (2006). Absorption and metabolism of conjugated alpha-linolenic acid given as free fatty acids or triacylglycerols in rats. *Nutrition & Metabolism*, 3, art. no. 8.

Poirier, H., Shapiro, J. S., Kim, R. J. y Lazar, M. A. (2006). Nutritional supplementation with *trans*-10, *cis*-12-conjugated linoleic acid induces inflammation of White adipose tissue. *Diabetes*, 55, 1634–1641.

Pouliot, Y. y Gauthier, S. F. (2006). Milk growth factors as health products: some technological aspects. *International Dairy Journal*, 16, 1415-1420.

Proteggente, A. R., Pannala, A. S., Paganga, G., van Buren, L., Wagner, E., Wiseman, S., van de Put, F., Dacombe, C. y Rice-Evans, C. A. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*, 36, 217-233.

Puupponen-Pimiä, R., Aura, A. M., Oksman-Caldentey, K. M., Myllärinen, P., Saarela, M., Mattila-Sandholm, T. y Poutanen, K. (2002). Development of functional ingredients for gut health. *Trends in Food Science and Technology*, 13, 3-11.

-Q-

Qu, W., Breksa III, A. P., Pan, Z. y Ma, H. (2012). Quantitative determination of major polyphenol constituents in pomegranate products. *Food Chemistry*, 132, 1585-1591.

-R-

Ramaswamy, H. S. y Basak, S. (1992). Pectin and raspberry concentrate effects on the rheology of stirred commercial yoghurt. *Journal of Food Science*, 57(2), 357-360.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A. y Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry*, 47, 4718-4723.

Rastegar, S., Rahemi, M., Baghizadeh, A. y Gholami, M. (2012). Enzyme activity and biochemical changes of three date palm cultivars with different softening pattern during ripening. *Food Chemistry*, 134, 1279-1286.

Resa, S. (2012). Alimentos que funcionan. *Distribución y Consumo*, Sept-Oct, 34-41.

Rissanen, H., Knekt, P., Jarvinen, R., Salminen, I. y Hakulinen, T. (2003). Serum fatty acids and breast cancer incidence. *Nutrition and Cancer*, 45, 168–175.

Rodríguez-Alcalá, L. M., Braga, T., Malcata, F. X., Gomes, A. y Fontecha, J. (2011). Quantitative and qualitative determination of CLA produced by Bifidobacterium and lactic acid bacteria by combining spectrophotometric and Ag^{+}-HPLC techniques. *Food Chemistry*, 125, 1373-1378.

Rodríguez-Alcalá, L. M., Harte, F. y Fontecha, J. (2009). Fatty acid profile and CLA isomers content of cow, ewe and goat milks processed by high pressure homogenization. *Innovative Food Science and Emerging Technologies*, 10, 32-36.

Rodríguez-Guisado, I., Hernández, F., Melgarejo, P., Legua, P., Martínez, R. y Martínez, J. J. (2009). Chemical, morphological and organoleptical characterisation of five Spanish quince tree clones (*Cydonia oblonga* Miller). *Scientia Horticulturae*, 122, 491-496.

Rogelj, I. (2000). Milk, dairy products, nutrition and health. *Food Technology and Biotechnology*, 38, 143-147.

Ross, H. A., McDougall, G. J. y Stewart, D. (2007). Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. *Phytochemistry*, 68, 218-228.

-S-

Sánchez-Moreno, C., Larrauri, J. A. y Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic compounds. *Food Research International*, 32, 407-412.

Sarkar, F. H. y Li, Y. (2002). Mechanisms of cancer chemoprevention by soy isoflavone genistein. *Cancer and Metastasis Reviews*, 21, 265-280.

Schoeller, D.A., Watras, A.C. y Whigham, L. D. (2009). A meta-analysis of the effects of conjugated linoleic acid on fat-free mass in humans. *Applied Physiology, Nutrition, and Metabolism*, 34, 975–978.

Ścibisz, I., Ziarno, M., Mitek, M. y Zaręba, D. (2012). Effect of probiotic cultures on the stability of anthocyanins in blueberry yoghurts. *LWT - Food Science and Technology*, 49, 208-212.

Sellappan, S., Akoh, C. C. y Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*, 50, 2432-2438.

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E. y Pérez-Álvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, 25(1), 13-21.

Serafeimidou, A., Zlatanos, S., Laskaridis, K. y Sagredos, A. (2012). Chemical characteristics, fatty acid composition and conjugated linoleic acid (CLA) content of traditional Greek yogurts. *Food Chemistry*, 134, 1839-1846.

Serefko, A., Szopa, A., Wlaź, P., Nowak, G., Radziwoń-Zaleska, M., Skalski, M., Poleszak, E. (2013). Magnesium in depression. *Pharmacological Reports*, 65(3), 547-554.

Serra, M., Trujillo, A. J., Guamis, B. y Ferragut, V. (2009). Flavour profiles and survival starter cultures of yoghurt produced from high-pressure homogenized milk. *International Dairy Journal*, 19, 100-106.

Sharma, R. (2005). Market trends and opportunities for functional dairy beverages. *The Australian Journal of Dairy Technology*, 60(2), 195-198.

Sharma, R., Joshi, V. K. y Rana, J. C. (2011). Nutritional composition and processed products of quince (*Cydonia oblonga* Mill.). *Indian Journal of Natural Products and Resources*, 2(3), 354-357.

Shinomiya, F., Hamauzu, Y. y Kawahara, T. (2009). Anti-allergic effect of a hot-water extract of quince (*Cydonia oblonga*). *Bioscience, Biotechnology and Biochemistry*, 78(8), 1773-1778.

Shireman, R. (2003). Essential fatty acids. En: Encyclopaedia of Food Science and Nutrition. 2nd Edition. Ed. Benjamín Caballero. Elsevier Science, London. pp: 2169-2176.

Silva, B. M., Andrade, P. B., Ferreres, F., Seabra, R. M., Oliveira, M. B. P. P. y Ferreira, M. A. (2005). Composition of quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids. *Natural Product Research*, 19(3), 275-281.

Silva, B. M., Andrade, P. B., Gonçalves, A. C., Seabra, R. M., Oliveira, M. B. y Ferreira, M. A. (2004b). Influence of jam processing upon the contents of phenolics, organic acids and free amino acids in quince (*Cydonia oblonga* Miller). European Food Res. Technol, 218, 385-389.

Silva, B. M., Andrade, P. B., Valentao, P., Ferreres, F., Seabra, R. M. y Ferreira, M. A. (2004a). Quince (*Cydonia oblonga* Miller) fruit (pulp, peel and seed) and jam: antioxidant activity. *Journal of Agricultural and Food Chemistry*, 52, 4705-4712.

Silva, M. R., Dias, G., Ferreira, C. L. L. F., Franceschini, S. C. C. y Costa, N. M. B. (2008). Growth of preschool children was improved when fed an iron-fortified fermented milk beverage supplemented with *Lactobacillus acidophilus*. *Nutrition Research*, 28, 226-232.

Silveira, M.-B., Carraro, R., Monereo, S. y Tébar, J. (2007). Conjugated linoleic acid (CLA) and obesity. *Public Health Nutrition*, 10(10A), 1181-1186.

Singh, G. y Muthukumarappan, K. (2008). Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. *LWT-Food Science and Technology*, 41, 1145-1152.

Singh, G., Arora, S., Sharma, G. S., Sindhu, J. S., Kansal, V. K. y Sangwan, R. B. (2007). Heat stability and calcium bioavailability of calcium-fortified milk. *LWT-Food Science and Technology*, 40, 625-631.

Singh, N. y Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85, 611-616.

Singlenton, V. L. y Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enolology and Viticulture*, 16, 144-158.

Slačanac, V., Božanić, R., Hardi, J., Szabó, J. R., Lučan, M. y Krstanović, V. (2010). Nutritional and therapeutic value of fermented caprine milk. *International Journal of Dairy Technology*, 63, 171-189.

Smedman, A. y Vessby, B. (2001). Conjugated linoleic acid supplementation in humans-metabolic effects. *Journal of Nutrition*, 36, 773-781.

UNIVERSITAS Miguel Hernández

Song, H. J., Grant, I., Rotondo, D., Mohede, I., Sattar, N., Heys, S. D. y Wahle, K. W. (2005). Effect of CLA supplementation on immune function in young healthy volunteers. *European Journal of Clinical Nutrition*, 59, 508–517.

Spanos, G. A., Wrolstad, R. E. y Heatherbell, D. A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry*, 38, 1572-1579.

Spormann, T. M., Albert, F. W., Rath, T., Dietrich, H., Will, F., Stockis, J. P., Eisenbrand, G. y Janzowski, C. (2008). Anthocyanin/polyphenolic-rich fruit juice reduces oxidative cell damage in an intervention study with patients on hemodialysis. *Cancer Epidemiology, Biomarkers & Prevention*, 17, 3372-3380.

Staffolo, M. D., Bertola, N., Mertino, M. y Bevilacqua, Y. A. (2004). Influence of dietary fibre addition on sensory and rheological properties of yogurt. *International Dairy Journal*, 14(3), 263-268.

Starling S. (2013). Word's fastest growing functional food in 2013. www.dairyreporter.com/content/view/print/839411 consultado el 17 de diciembre de 2013, William Reed Busssines Media.

Starling S. (2014). Polyphenol use rises 69% in 5 years (but do people get it?. http://www.nutraingredients.com/Consumer-Trends/Polyphenol-use-rises-69-in-5-years-but-do-people-get-it, consultado el 24 de enero de 2014, William Reed Busssines Media.

Steffe, J.F. (1996). Rheological methods in food process engineering. East Lansing, Michigan: Freeman Press. pp. 295-349.

Stowe, C. B. (2011). The effects of pomegranate juice consumption on blood pressure and cardiovascular health. *Complementary Therapies in Clinical Practice*, 17(2), 113-115.

Sun, C. Q., O'Connor, C. J. y Roberton, A. M. (2002a). The antimicrobial properties of milkfat after partial hydrolysis by calf pregastric lipase. *Chemico-Biological Interactions*, 140, 185-198.

Sun, J., Chu, Y.-F., Wu, X. y Liu, R. H. (2002b). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, 50, 7449-7454.

Svensson, M., Hakansson, A., Mossberg, A. K., Linse, C. y Svanborg, C. (2000). Conversion of β-lactoglobulin to a protein inducing apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, 97(8), 4221-4226.

Szychowski, P. J., Munera-Picazo, S., Szummy, A., Carbonell-Barrachina, A. A. y Hernández, F. (2014). Quality parameters, bio-compounds, antioxidant activity and sensory attributes of Spanish quinces (*Cydonia oblonga* Miller). *Sciencia Horticulturae*, 165, 163-170.

-T-

Tamime, A. Y., Wszolek, M., Božanić, R. y Özer, B. (2011). Popular ovine and caprine fermented milks. *Small Ruminant Research*, 101, 2-16.

Tamime, A. Y. y Robinson, R. K. (2007). *Yoghurt science and technology*. Cambridge: Woodhead Publishing Ltd.

Tamime, A. Y. y Deeth, H. C. (1980). Yoghurt: technology and biochemistry. *Journal of Food Protection*, 43, 151–187.

Tang, S., Sheehan, D., Buckley, D. J., Morrissey, P. A. y Kerry, J. P. (2001). Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *International Journal of Food Science and Technology*, 36, 685–692.

Tehranifara, A., Zareia, M., Nematia, Z., Esfandiyaria, B. y Vazifeshenasb, M. R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, 126, 180–185.

Teixeira da Silva, J. A., Rana, T. S., Narzary, D., Verma, N., Meshram, D. T. y Ranade, S. A. (2013). Pomegranate biology and biotechnology: a review. *Scientia Horticulturae*, 160, 85-107.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, 115(3), 873–877.

Thiébaut, A., Chajés, V., Gerber, M., Boutron-Ruault, M. C., Joulin, V., Lenoir, G., Berrino, F., Riboli, E., Bénichou, J. y Clavel-Chapelon, F. (2009). Dietary intakes of ω-6 and ω-3 polyunsaturated fatty acids and the risk of breast cancer. *International Journal of Cancer*, 124, 924-931.

Thom, E., Wadstein, J. y Gudmundson, O. (2001). Conjugated linoleic acid reduces body fat in healthy exercising humans. *Journal of International Medical Research*, 29, 392-396.

Thomasset, S., Teller, N., Cai, H., Marko, D., Berry, D. P., Steward, W. P. y Gescher, A. J. (2009). Do anthocyanins and anthocyanidins, cancer chemopreventive pigments in the diet, merit development as potential drugs? *Cancer Chemotherapy and Pharmacology*, 64, 201-211

Thormar, H. y Hilmarsson, H. (2007). The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chemistry and Physics of Lipids*, 150, 1-11.

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C. y Saija, A. (2010). Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie*, 92(9), 1115-1122.

Tomás-Barberán, F. y Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, 81, 853-876.

Toor, R. K. y Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International*, 38, 487-494.

Tormo, M. e Izco, J. M. (2004). Alternative reversed-phase high-performance liquid chromatography method to analyse organic acids in dairy products. *Journal of Chromatography A,* 1033, 305-310.

Tricon, S., Burdge, G. C., Kew, S., Banerjee, T., Russell, J. J., Grimble, R. F., Williams, C. M., Calder, P. C. y Yaqoob, P. (2004). Effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on immune cell function in healthy humans. *The American Journal of Clinical Nutrition*, 80, 1626–1633.

Tsuzuki, T., Kambe, T., Shibata, A., Kawakami, Y., Nakagawa, K. y Miyazawa, T. (2007). Conjugated EPA activates mutant p53 via lipid peroxidation and induces p53-dependent apoptosis in DLD-1 colorectal adenocarcinoma human cells. *Biochimica et Biophysica Acta*, 1771(1), 20–30.

Tsuzuki, T., Kawakami, Y., Nakagawa, K. y Miyazawa, T. (2006). Conjugated docosahexaenoic acid inhibits lipid accumulation in rats. *The Journal of Nutritional Biochemistry*, 17(8), 518-524.

Tsuzuki, T., Kawakami, Y., Suzuki, Y., Abe, R., Nakagawa, K. y Miyazawa, T. (2005). Intake of conjugated eicosapentaenoic acid suppresses lipid accumulation in liver and epididymal adipose tissue in rats. *Lipids*, 40(11), 1117-1123.

Turpeinen, A. M., Ylonen, N., von Willebrand, E., Basu, S. y Aro, A. (2008). Immunological and metabolic effects of *cis*-9, *trans*-11-conjugated linoleic acid in subjects with birch pollen allergy. *The British Journal of Nutrition*, 100, 112–119.

-U-

Uzzan, M. y Labuza, T. P. (2004). Critical issues in R&D of soy isoflavone-enriched foods and dietary supplements. *Journal of Food Science*, 69(3), CRH77-CRH86.

-V-

van der Sluis, A. A., Dekker, M. y van Boekel, M. A. J. S. (2005). Activity and concentration of polyphenolic antioxidants in apple juice. 3. Stability during storage. *Journal of Agricultural and Food Chemistry*, 53, 1073-1080.

van Wijlen, R. P. J. y Colombani, P. C. (2010). Grass-based ruminant production methods and human bioconversion of vaccenic acid with estimations of maximal dietary intake of conjugated linoleic acid. *International Dairy Journal*, 20, 433-448.

Viladomiu, M., Hontecillas, R., Lu, P. y Bassaganya-Riera, J. (2013). Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. *Evidence-Based Complementary and Alternative Medicine*, 2013, art. no. 789764.

Vincent, R. P. y le Roux, C. W. (2007). New agents in development for the management of obesity. *International Journal of Clinical Practice*, 61(12), 2103-2112.

Viuda-Martos, M., Fernández-López, J. y Pérez-Álvarez, J. A. (2010a). Pomegranate and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety*, 9, 635-654.

Viuda-Martos, M., Fernández-López, J. y Pérez-Álvarez, J.A. (2009). Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory

characteristics of a bologna sausage. *Innovative Food Science and Emerging Technologies*, 10, 655-660.

Viuda-Martos, M., Fernández-López, J., Sendra Nadal, E., Sayas, M. E., Navarro, C., Sánchez-Zapata, E. y Pérez-Álvarez, J.A. (2008). Caracterización físico-química de las aguas de lavado obtenidas en el proceso de reutilización de un co-producto cítrico. Proceedings of II congreso CESIA, (p-263). Barcelona. España.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. y Pérez-Álvarez, J. A. (2010c). Effect of adding citrus fibre washing water and rosemary essential oil on the quality characteristics of a bologna sausage. *LWT-Food Science and Technology*, 43, 958-963.

Viuda-Martos, M., Ruiz-Navajas, Y., Sánchez-Zapata, E., Fernández-López, J. y Pérez-Álvarez, J. A. (2010b). Antioxidant activity of essential oils of five spice widely used in a Mediterranean diet. *Flavour and Fragance Journal*, 25, 13-19.

Viuda-Martos, Ruiz-Navajas, Y., Fernández-López, J., Sendra, E., Sayas-Barberá, E. y Pérez-Álvarez, J. A. (2011). Antioxidant properties of pomegranate (*Punica granatum* L.) bagasses obtained as co-product in the juice extraction. *Food Research International*, 44(5), 1217-1223.

Volpe, S. L. (2013). Magnesium in disease prevention and overall health. *Advances in nutrition (Bethesda, Md.)*, 4(3), 378S-383S.

Voorrips, L. E., Brants, H. A., Kardinaal, A. F., Hiddink, G., van den Brandt, J. y Goldbohm, P. A. (2002). Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. *The American Journal of Clinical Nutrition*, 76, 873–882.

-W-

Wahle, K. W., Heys, S. D., Rotondo, D. (2004). Conjugated linoleic acids: are they beneficial or detrimental to health?. *Progress in Lipid Research*, 43(6), 553–587.

Wakabayashi, H., Yamauchi, K. y Takase, M. (2006). Lactoferrin research, technology and applications. *International Dairy Journal*, 16(11), 1241-1251.

Wallace, T. C., Reider, C. y Fulgoni, V. L. (2013). Calcium and vitamin D disparities are related to gender, age, race, household income level, and weight classification but not vegetarian

status in the United States: analysis of the NHANES 2001-2008 data set. *Journal of the American College of Nutrition*, 32(5), 321-330.

Wallace, T. C. y Giusti, M. M. (2008). Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylated anthocyanins from *Berberis boliviana* L. as compared to other natural/synthetic colorants. *Journal of Food Science*, 73(4), C241-C248.

Wang, Y. y Proctor, S. D. (2013). Current issues surrounding the definition of *trans*-fatty acids: implications for health, industry and food labels. *British Journal of Nutrition*, 110, 1369-1383.

Wang, W. D. y Xu, S. Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. Journal of Food Engineering, 82 (3), 271-275.

Wang, Q., Allen, J. C. y Swaisgood, H. E. (1997). Binding of vitamin D and cholesterol to β-lactoglobulin. *Journal of Dairy Science*, 80, 1054-1059.

Weiler, H., Austin, S., Fitzpatrick-Wong, S., Nitschmann, E., Bankovic-Calic, N., Mollard, R., Aukema, H. y Ogborn, M. (2004). Conjugated linoleic acid reduces parathyroid hormone in health and in polycystic kidney disease in rats. *The American Journal of Clinical Nutrition*, 79, 1186S–1189S.

Whigham, L. D., Watras, A. C. y Schoeller, D. A. (2007). Efficacy of conjugated linoleic acid for reducing fat mass: ameta-analysis in humans. *The American Journal of Clinical Nutrition*, 85, 1203–1211.

Wijeratne, S. S. K., Amarowicz, R., Shahidi, F. (2006). Antioxidant activity of almonds and their by-products in food model systems. *JAOCS, Journal of the American Oil Chemists' Society*, 83(3), 223-230.

Wojdylo, A., Oszmiański, J. y Bielicki, P. (2013). Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (*Cydonia oblonga* Miller) varieties. *Journal of Agricultural and Food Chemistry*, 61, 2762-2772.

Wojdylo, A., Oszmiański, J. y Bober, I. (2008). The effect of addition of chokeberry, flowering quince fruits and rhubarb juice to strawberry jams on their polyphenol content, antioxidant activity and colour. *European Food Research and Technology*, 227(4), 1043-1051.

World Health Organization (WHO) (2013a). OMS | Enfermedades cardiovasculares. http://www.who.int/mediacentre/factsheets/fs317/es/. Última consulta: 14/11/2013.

World Health Organization (WHO) (2013b). Obesidad y sobrepeso. Nota descriptiva N°311. http://www.who.int/mediacentre/factsheets/fs311/es/. Última consulta: 14/11/2013.

Woyengo, T. A., Ramprasath, V. R. y Jones, P. J. H. (2009). Anticancer effects of phytosterols. *European Journal of Clinical Nutrition*, 63, 813-820.

Wrolstad, R. E. (1976). Color and pigments analyses in fruit products. Agricultural Experiment Station, Oregon State University, Corvallis, Oregon. Station Bulletin. Reprinted 1993, 624, 1-17.

-X-

Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C. y Yamamoto, K. (2011). Interaction of dietary polyphenols with bovine milk proteins: molecular structure-affinity relationship and influencing bioactivity aspects. *Molecular Nutrition & Food Research*, 55, 1637-1645.

Xu, S., Boylston, T. D. y Glatz, B. A. (2004). Effect of lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. *Journal of the American Oil Chemists' Society*, 81, 589-595.

Xu, S., Boylston, T. D. y Glatz, B. A. (2005). Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. *Journal of Agricultural and Food Chemistry*, 3, 9064-9072.

-Y-

Yang, C. S., Maliakal, P. y Meng, X. (2002). Inhibition of carcinogenesis by tea. *Annual Review of Pharmacology and Toxicology*, 42, 25-54.

Yang, M. y Cook, M.E. (2003). Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-alpha production, and modifies splenocyte cytokines production. *Experimental Biology and Medicine*, 228, 51–58.

Yu, Y., Correll, P. H. y Vanden Heuvel, J. P. (2002). Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. *Biochimica et Biophysica Acta*, 1581, 89–99.

Capítulo 7: Publicaciones

7.1. PUBLICACIONES

PUBLICACIÓN 1

Production of low-fat yogurt with quince (*Cydonia oblonga* Mill.) scalding water

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Production of low-fat yogurt with quince (*Cydonia oblonga* Mill.) scalding water

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ABSTRACT

Quince scalding water is rich in phenolic compounds and flavonoids which provide interesting antioxidant properties, and also contain organic acids and sugars. The aim of this study was to evaluate the direct use of quince scalding water for set style yogurt production. The addition of quince scalding water provided color changes and reduced yogurt sensory scores. Quince scalding water had inhibitory effect against lactic acid bacteria, probably due to its high content in polyphenols. As a consequence, quince scalding water enriched yogurts had higher pH and lower lactic acid content compared to control yogurts. Such changes are reflected in their rheological and textural properties: soft yogurts of higher deformability and lower elastic behavior and viscosity. Future research on the addition of quince scalding water to other foods, or the study of their antibacterial or antioxidant properties would be of great interest.

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1. Introduction

Waste water could result from several steps in the food industry like the cleaning process, the step of blanching fruits or vegetables, or as secondary by-product such as washing water during the extraction of dietary fiber. The possibility of successfully including these waste water by-products in the human food industry would help in enhancing the economic development quince producers and processors.

Quince fruit (*Cydonia oblonga* Miller) is not appreciated fresh because of pulp hardness, bitterness and astringency. But when ripe it is highly demanded for processing 'marmalade', jams, jelly and cakes (Silva et al., 2002; Silva et al., 2005). Rodríguez-Guisado et al. (2009) determined the average values for the chemical characterization of five quince fruits collected in Southeastern Spain showing high water content (76.72 g/100 g), high crude fiber content (5.33 g/100 g), and low-fat content (1.95 g/100 g). Quince (like apple and pear) is classified into the Roseaceae family and their well-established beneficial properties to human health were found mainly related to their phenolic content. Fattouch et al. (2008) reported that quince pulp extract showed a superior phenolic content (66.95 mg/100 g fresh weight) than apple pulp (27.44 mg/100 g fresh weight) and pear (24.38 mg/100 g fresh weight). So the fact that quince fruits are a source of sugars (mainly fructose, glucose, and sucrose) and phenolic compounds with antioxidant activity suggests that it is likely that a small portion of these compounds become part of the scalding water when they are processed. Quince healthy properties have been explored by several authors, a recent study has proposed the use of quince hot water, scalding water, as a functional food for its anti-allergic effects on type I allergic symptoms proved in mice and *in vitro* cells (Shinomiya, Hamauzu, & Kawahara, 2009). However, the reuse of water in the food industry has limits, as has been reviewed by Casani, Rouhany, and Knøchel (2005). The type of water that we are using in the present study is classified as water for direct recycling for non-food uses and cleaning (Casani et al., 2005), however, given the interesting compounds that contains and the references to its health benefits we considered to present a simple approach by directly using such water in foods.

Fermented milk products already have a positive healthy image due to the beneficial action of its viable bacteria and yogurt already have a record as being healthful (Heller, 2001). The addition of antioxidant food ingredients such as green tea and lemon, strawberry pulp and vitamin E was been also tested on dairy products (Jiménez, Murcia, Parras, & Martínez-Tomé, 2008). The purpose of incorporating ingredients known to have antioxidant activity is to increase the functionality and antioxidant activity of these foodstuffs and in this way to improve consumer's protection against pathologies related with free radicals (Jiménez et al., 2008).

The aim of this study was to evaluate the direct use of scalding water from quince fruits scalding in a set style yogurt on yogurt quality during 28 days of refrigerated storage.

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2. Materials and methods

2.1. Materials

Quince fruits were directly collected from the quince grove of the Miguel Hernandez University (Orihuela, Alicante, Spain). Quince fruits were separately scalded at 100 °C for 3 min and immediately divided into solids (seeds, pulp, peels) and liquid. For scalding, quince:water ratio was 1:1. Seeds and peels were handy removed, the flesh part was crushed to obtain a paste. Scalding water was filtered through a cotton gauze and frozen stored until use. For best knowledge the scalding water (quince scalding water, QSW) and the resulting quince fruit paste (QFP) were analyzed to compare how many bioactive compounds in the pulp and skin were released to the scalding water.

For all the study the same batch of skim milk powder was used (34 g/100 g protein, 52 g/100 g lactose, 1 g/100 g fat, 6.8 g/100 g ash, 5.2 g/100 g moisture; Central Lechera Asturiana, CAPSA, Granada-Siero, Spain). Commercial starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Ezal© MY900, Rhodia Food-Danisco A/S, Sassenage, France) were used at the concentrations prescribed by the suppliers. Only for batches prepared for sensory analysis 8 g/100 g of sucrose was added to each mix (prior to pasteurization) to sweeten the yogurt.

2.2. Yogurt manufacture

Control and quince yogurt were manufactured. Skim milk powder was reconstituted with deionised water which is a common industrial practice (Augustin, Clarke, & Craven, 2003) and quince scalding water (QSW) at 15 g/100 mL for control and quince yogurt respectively. Reconstituted skim milk (RSM) was pasteurized in a water bath at 80 °C for 30 min, followed by immersion in ice-water baths to cool down to 43 °C, at this point the starter culture was added and gently shaken. The inoculated mix was poured into brand new 100 mL cups of yogurt and incubated at 43 °C to reach pH 4.7, and then cooled down to 4 °C.

2.3. Raw material analysis

2.3.1. pH and °Brix

pH and soluble solids (°Brix) were determined at 20 °C using a pH-meter and a refractometer respectively.

2.3.2. Chemicals and extraction process

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ferrozine, Folin-Ciocalteu's reagent, gallic acid, iron (III) chloride, iron (II) chloride, trichloroacetic acid (TCA) and Trolox were from Sigma Chemical Company (Germany). Dibasic potassium phosphate, sodium carbonate and dibasic sodium phosphate were from Merck (Darmstadt, Germany). Potassium hexacyanoferrate was from Fluka BioChemika (Germany). The solvent used for the extraction process was methanol of HPLC ultra-gradient grade, supplied by Merck.

For the extraction process two hundred milligrams of quince fruit paste (QFP) were extracted for 2 h with 2 mL of methanol/H_2O (50:50 v/v) at room temperature on an orbital shaker set at 600 rpm. The mixture was centrifuged at 1000*g* for 15 min, and the supernatant was decanted. Quince scalding water was directly used for analysis.

2.3.3. Measurements of total phenol content

The total phenol content (TPC) was determined using Folin-Ciocalteu's reagent (Viuda-Martos, Ruiz Navajas, Sánchez Zapata & Fernández-López, 2010).

2.3.4. Measurements of total flavonoid content

For total flavonoid content (TFC), the method based on Blasa et al. (2005).

2.3.5. Determination of antioxidant activity

2.3.5.1. 2,2′-Diphenyl-1-picrylhydrazyl radical scavenging method. *2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method* was run according to Viuda-Martos et al. (2010)

2.3.5.2. Ferric reducing antioxidant power. *Ferric reducing antioxidant power* (FRAP) of the QFP extracts (100 g/L) or scalding waters was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986).

2.3.5.3. Ferrous ion-chelating ability assay. *Ferrous ion-chelating ability assay* (FIC) assay was carried out according to the method of Singh and Rajini (2004).

2.3.6. Organic acids and sugars analysis

The content of organic acids and sugars were determined as follows: 5 g samples with 10 mL ultrapure water acidified with 0.1 g/100 g phosphoric acid H_3PO_4 were homogenized for 20 s at 13500 rpm and centrifuged for 20 min at 15000 rpm. Triplicate extractions were obtained from each sample. The supernatants fluids were filtered through 0.45 μm membrane filter (Millipore). Ten microliter samples were injected in a cation exchange column (Supelcogel C-610 H, 300 × 7.8 mm, Supelco, Bellefonte) with a pre-column (Supelguard-H, 50 × 4.6 mm, Supelco, Bellefonte), using 0.1 g/100 g H_3PO_4 as mobile phase, operating flow rate of 0.5 mL/min. A Hewlett Packard HP-1100 instrument (Woldbronn, Germany) coupled with two detectors: DAD G1315A (set at 210 nm) and RID G-1362 A was used. Standards of organic acids, monosaccharides and oligosaccharides were obtained from Supelco. Samples were run at 30 °C and the run time was 30 min (Doughty, 1995). Peaks were identified by comparison with retention time of the standards, and quantified by regression formula obtained with the standards.

2.4. Yogurt analysis

2.4.1. Physicochemical analysis

2.4.1.1. Color measurements. The CIE LAB color space of yogurts was studied. Color determinations were made at 12 ± 2 °C by means of a Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer, with a liquid accessory CR-A70 (Minolta Camera Co., Osaka, Japan), with illuminant D_{65} and an observer of 10°. Six measures per sample were taken.

2.4.1.2. Texture analysis. Penetration test was performed with a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, England) and a 5-kg load cell was used. Constant speed penetration tests were performed directly on cylindrical containers (4.5 cm diameter × 4 cm height). All instrumental texture analyses were conducted at 7 °C. This is a 'destructive' test as no structure recovery is allowed. A cylindrical probe 10 mm diameter ebonite (P-10) was introduced 15 mm into the samples at a speed of 1 mm/s. From the force-versus-time curves, values for the maximum force (N) were calculated as force at a distance of 15 mm (F_{max}). Triplicate measures for each yogurt were performed.

2.4.2. Rheological measurements

The rheological parameters were measured by oscillatory testing, in triplicate at 7 °C by means of a rheometer Rheostress 600 (Haake, Karlsruhe, Germany). Tests were performed on slices of 1.2 ± 0.2 mm thickness and 35 mm diameter obtained using a sharp blade. It was verified that all samples were within the range of linear

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viscosity. The geometry used was plate and plate with serrated platens (35 mm diameter, 1 mm gap). The measurements were conducted in triplicate. Frequency test was run: frequency sweep from 0.01 to 10 Hz at a constant stress of 0.4 Pa with three cycles of measurements in both tests.

2.4.3. Sensory evaluation

Panelists (36) were members of the staff and students of the Miguel Hernández University, Alicante, Spain. All sensory work was carried out in the sensory laboratory at the University, which fulfills requirements according to the International Standards (ISO, 1988). In all cases a panel of three experts defined the attributes to be evaluated in yogurts. Panelists accepted to taste the samples before the tests, and they were informed of the type of product being tested and asked about yogurt consumption habits. Tap water was provided between samples to cleanse the palate. White plastic cups with 40 mL of yogurt at 10 °C were provided. Two samples were evaluated in each session. A total of three sessions were run. A seven-point hedonic scale (from dislike extremely to like extremely) was used to rate the following parameters: appearance, creaminess, sweetness, astringency, sourness, flavor and overall acceptance.

2.4.4. Microbiological analysis

Microbial analyses of yogurts were run at days 1, 7, 14, 21 and 28 of cold storage. Streptococci were counted on M-17 agar (Scharlau, Barcelona, Spain) incubated aerobically at 37 °C for 24 h. Lactobacilli were enumerated using MRS agar (Cultimed, Panreac, Castellar del Vallés, Barcelona, Spain) under anaerobic incubation at 37 °C for 48 h. Counting of yeasts and moulds were done on Rose Bengal agar with Chloramphenicol (Scharlau, Barcelona, Spain) using the spread plate technique and plates were incubated under aerobic conditions at 28 °C for 5 days. Samples were analyzed in duplicate.

2.4.5. Organic acids and sugars analysis

Organic acids and sugars from yogurts were analyzed during cold storage, as previously described in Section2.3.6.

2.5. Statistical analysis

PASW Statistics 18 software package, SPSS Inc., IBM (Chicago, IL, USA) was used. General Linear Model procedure was tested to evaluate factors (water source and storage time effect on yogurt samples). Tukey's test was used for means comparison (95% confidence level). For sensory data of yogurts and scalding water samples one way anova test was used. The whole experiment was independently run in triplicate.

3. Results and discussion

3.1. Raw material characterization

3.1.1. pH and °Brix

The average pH was 3.92 for QFP. °Brix was 11.73 in QFP. Rodríguez-Guisado et al. (2009) reported a quince fruit pH ranging from 3.60 to 3.84 and total soluble solids (as °Brix) from 11.57 to 14.70. The pH of quince scalding water was 4.83 and the °Brix 3.53.

3.1.2. Organic acid and sugar profile in raw materials

Table 1 shows the profile of sugars and organic acids of quince paste and scalding water and reconstituted skim milk powder.

Fructose was the predominant sugar in either QFP or QSW followed by manitol and glucose in similar amounts. Main organic acid in QFP was acetic acid and in lower amounts formic, malic and succinic acids. QSW contained acetic and malic acids as predominant acids followed by tartaric, succinic and formic acids (Table 1). The high presence of acetic acid was due to quince was over-ripened; organic acids provide information about maturation stage of the fruit. Rodríguez-Guisado et al. (2009) reported that the predominant sugars in quince fruit cultivated in Southeastern Spain are fructose and glucose (7.95 and 5.00 g/100 g, respectively) and malic the main organic acid (0.78 g/100 g) followed by tartaric acid (0.22 g/100 g) and acetic acid (0.13 g/100 g). Overall, QSW was able to extract a great part of organic acids (mainly tartaric acid) and sugars from quince fruit. In this line, several authors have successfully recuperated water-soluble polysaccharides presents in hot water extracts from different fruits (Mandal et al., 2009; Yang et al., 2009) and sucrose from the liquid content of the solid wastes of the citrus juice industry by reverse osmosis (García, Gozálvez, & Lora, 2002). On the other hand, QFP presented nearly 4-fold higher total acidity and total soluble solids than QSW.

Table 1
Organic acids (mg/100 g) and sugars (g/100 g) profile of reconstituted skimmed milk and quince paste and scalding water.

	RSM	Quince	
		Paste	QSW
Organic acids (mg/100 g)			
Oxalic acid	3.60 (0.44)[d]	N.d.	N.d.
Citric acid	233.41^{c} (24.3)	84.72^{b} (2.73)	34.41^{a,b} (5.42)
Tartaric acid	400.31^{b} (43.3)	88.68^{a} (1.04)	74.71^{a} (5.57)
Malic acid	2.47^{a} (0.69)	303.73^{c} (12.63)	97.81^{b} (28.13)
Ascorbic acid	0.37^{a} (0.11)	10.40^{c} (0.11)	2.89^{b} (1.14)
Succinic acid	N.d.	270.14^{b} (22.16)	63.69^{a} (24.42)
Lactic acid	122.23 (3.93)	N.d.	N.d.
Formic acid	N.d.	318.97^{b} (39.89)	53.92^{a} (22.09)
Acetic acid	148.51^{b} (1.87)	684.60^{c} (49.29)	103.09^{a} (34.24)
Fumaric acid	3.36^{a} (0.12)	11.01^{b} (0.99)	1.54^{a} (1.54)
TOTAL ACIDITY (g/100 g)	0.91^{b} (0.07)	1.77^{c} (0.12)	0.43^{a} (0.12)
Sugars (g/100 g)			
Sucrose	N.d.	0.23^{b} (0.00)	0.06^{a} (0.01)
Glucose	N.d.	2.12^{b} (0.08)	0.60^{a} (0.15)
Fructose	N.d.	5.09^{b} (0.18)	1.42^{a} (0.36)
Manitol	N.d.	2.23^{b} (0.07)	0.62^{a} (0.15)
Lactose	6.40 (0.65)	N.d.	N.d.
TOTAL SUGARS (g/100 g)	6.40^{b} (0.65)	9.67^{c} (0.33)	2.70^{a} (0.67)

RSM: reconstituted skimmed milk, QSW: quince scalding water; N.d., not detected. $n = 9$.
Values with different superscript letters within the same line significantly differ (Tukey's test, $*P < 0.05$).
[d] Values in parentheses denote standard error.

Lactose was the main sugar in skim milk. Main organic acid identified in skim milk was tartaric acid followed by citric, acetic and lactic acids and small amounts of other organic acids (oxalic, fumaric, malic and ascorbic acids).

3.1.3. Total phenol and total flavonoid content

The total phenol and flavonoid contents of QFP and its scalding waters are presented in Table 2. As can be seen QFP contained higher amounts of phenolics and flavonoids than QSW. The presence of these compounds in scalding water is due to leaching during the scalding operation.

Quince (like apple and pear) is classified into the Roseaceae family and their well-established beneficial properties to human health were found mainly related to their phenolic content. Fattouch et al. (2008) reported that quince pulp aqueous acetone extract showed a superior phenolic content (66.95 mg/100 g fresh weight) than apple pulp (27.44 mg/100 g fresh weight) and pear (24.38 mg/100 g fresh weight). Furthermore, Silva et al. (2004) founded that antioxidant activity of quince pulp, peel and jam methanolic extracts were strongly correlated with TPC. Recently, Magalhaes et al. (2009) showed much higher contents of TPC (250 mg/100 g for a methanolic extract of quince pulp) than those of Fattouch et al. (2008).

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Table 2
Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of quince paste (QP) and its scalding water (QSW) at different concentrations (A = 2.5 g/100 g, B = 5 g/100 g, C = 7.5 g/100 g, D = 10 g/100 g).

Type of raw material	TPC (mg GAE[e]/L)	TFC (mg RE[f]/100 g)	DPPH Inhibition (50 per cent)					FRAP TEAC[g] (mM Trolox/L)			
			A	B	C	D	$^{h}IC_{50}$	A	B	C	D
QP	1595.67^{B} (7.27)[i]	459.86^{B} (23.21)	4.22^{aA} (0.61)	$6.81^{a,bA}$ (0.61)	8.72^{bA} (0.08)	12.69^{cA} (1.00)	19.61	0.07^{aA} (0.00)	0.10^{bA} (0.00)	0.15^{cA} (0.00)	0.19^{dA} (0.01)
QSW	410.80^{A} (15.88)	70.40^{A} (2.90)	9.74^{aB} (0.23)	14.24^{aB} (0.14)	21.32^{bB} (2.07)	22.70^{bB} (0.05)	24.21	0.24^{aB} (0.06)	0.46^{bB} (0.03)	0.57^{bB} (0.02)	0.93^{cB} (0.04)

Values with different small superscript letters within the same line significantly differ (Tukey's test, $*P < 0.05$); Values with different capital superscript letters within the same column significantly differ (Tukey's test, $*P < 0.05$). $n = 9$.
[e] Gallic acid equivalent.
[f] Rutin equivalent.
[g] TEAC, Trolox equivalent antioxidant capacity.
[h] IC_{50}, concentration (g/100 g) for a 50 per cent. inhibition.
[i] Values in parentheses denote standard error.

Different amounts of total phenolics have been reported in several food by-products such as juice by products from apple (46.00 mg GAE/g), strawberry (39.39 mg GAE/g) and pear (12.90 mg GAE/g), among others (Peschel et al., 2006). QSW contained higher levels of TPC than the reported in such juices, and the reported flavonoid content was also high.

3.1.4. Antioxidant activities

Table 2 shows the concentrations required to scavenge DPPH radical and the scavenging values as inhibition (50 per cent). QSW and QFP showed good radical scavenging effect. In the DPPH assay, the higher the antioxidant activity the lower the IC_{50}. Viuda-Martos et al. (2010) reported IC_{50} values of 0.042 per cent and 0.053 per cent for ascorbic acid and butylated hydroxytoluene (BHT) as positive controls. Fattouch et al. (2008) reported that quince peels aqueous acetone extracts showed the greatest antioxidant effect on DPPH radicals (57 per cent of inhibition, corresponding to 0.44 mM trolox equivalents) and also quince pulp showed the strongest effect over pear, apple and quince pulps extracts analyzed.

Table 4 also shows the results of the FRAP assay. Quince paste, at all the concentrations analyzed, showed good ferric reducing capacity in terms of Trolox concentrations. The inhibition was concentration-dependent. Although both QSW and QFP showed antioxidant activity by DPPH and FRAP assays, no iron chelating ability was detected on quince paste or scalding water. Other water extracts from fruits like figs (Yang et al., 2009) and Doum palm fruit (Hsu, Coupar, & Ng, 2006) have demonstrated to have potent antioxidant activity.

3.2. Characterization of yogurt with quince scalding water

3.2.1. Acidification process

The enrichment of yogurts with QSW causes an initial pH reduction ($P < 0.05$) (Fig. 1) due to the acidity of the QSW. QSW provides a low pH together with high formic acid content (Table 1) that may stimulate the growth of lactobacilli (Robinson, 2003).

3.2.2. Physicochemical analysis of yogurts

3.2.2.1. Color. The addition of QSW had no effect ($P > 0.05$) on color coordinates L* (84.27 ± 1.11 for control yogurt and 83.07 ± 2.48 for quince yogurt) and b* (5.34 ± 0.16 for control yogurt and 6.15 ± 0.49 for quince yogurt), but significantly increased a* (to −2.74 ± 0.06 for control yogurt from −2.21 ± 0.23 for quince yogurt) as QSW had a light yellowish color.

3.2.2.2. Texture. QSW yogurts had significantly ($P < 0.05$) lower F_{max} values (20.52 ± 0.71 N) than control yogurt (32.56 ± 1.04 N),

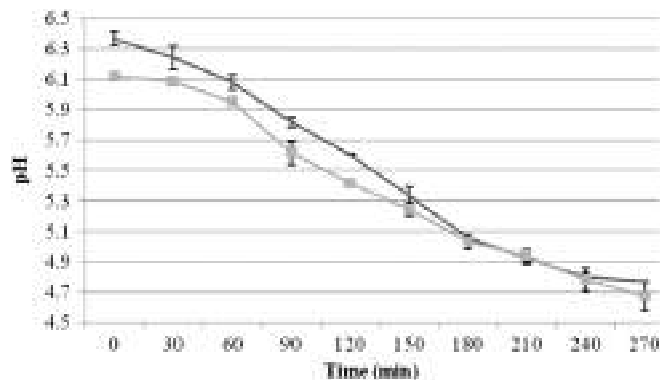

Fig. 1. Acidification curves of control yogurts (▬▬) and quince enriched yogurts (▬■▬). Data are the average ($n = 9$) ± standard error (Tukey's test, $*P < 0.05$).

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even when QSW provided some soluble solids. In yogurt, the strength of the protein network increases along with the increase of lactic acid and exopolysaccharides released by lactic acid bacteria (Lubbers, Decourcelle, Vallet, & Guichard, 2004). So, the softening effect of QSW addition may have been due to the reduced populations of lactobacilli observed in QSW yogurts (Fig. 3b). QSW addition to skim milk powder induced a sudden pH decrease (Fig. 1) of 0.3 units which may have affected the casein structure during pasteurization and further set gel formation.

3.2.3. Rheology of set style yogurt enriched with quince scalding water

Oscillation tests allow the calculation of elastic modulus and loss modulus, G' and G'', which are related to energy stored and released, respectively, per deformation cycle, whereas tan (δ) is associated with the degree of viscoelasticity of the sample (Singh & Muthukumarappan, 2008). Set style yogurts have flow properties that are characteristic of a non-Newtonian, weakly viscoelastic fluid with a highly time-dependent behavior (Ares, Paroli, & Harte, 2006). The studied yogurts remained in the linear viscoelastic region over the whole range of frequencies tested (0.01–1 Hz). G', G'', η^*, tan (δ) and γ at 0.1 Hz were selected to run statistical analysis of the data (Table 3).

Coinciding with the observations of Sendra et al. (2010) the yogurts showed a predominantly elastic behavior ($G' > G''$) over the whole range of frequencies tested, which corresponds closely to that of a true gel. In general, moduli (G' and G'') increased with increased frequency. Our experimental values for the tangent of the phase shift of phase angle (tan δ) ranged from 0.210 to 0.246 which pointed to a concentrated amorphous polymer (Steffe, 1996, pp. 294–349) and a low value of tan (δ) indicates that the gels had a predominant elastic character (more solid like). The linear decrease of the complex viscosity (data not shown) corresponds to a typical shear thinning profile.

The type of yogurt significantly affected rheological parameters ($P < 0.05$). G', G'', η^* decreased with the addition of QSW, whereas tan (δ) and γ increased with its presence. Storage time only affected ($P < 0.05$) tan (δ) and γ, both decreased with storage time, pointing to a hardening of the structure, probably related to the increase in lactic acid and exopolysaccharides released by lactic acid bacteria (Table 4). Under the same stress and frequency conditions, the strain (γ) achieved in QSW yogurts was higher than that of control yogurts, pointing to a softer structure as has been also detected by the penetration test.

3.2.4. Sensory analysis

There were significant differences in appearance, astringency, flavor and overall acceptability, which were best scored in control yogurts than in QSW yogurts (Fig. 2). Creaminess, sweetness and sourness were adjudged to be very similar in all samples.

3.2.5. Evolution of pH and microbial counts during cold storage

Higher pH values were observed ($P < 0.05$) in QSW than in control yogurts. pH decreases gradually (Fig. 3a) during the storage period, presumably due to continued fermentation by the lactic acid bacteria (Dave & Shah, 1997).

Average initial microbial counts on yogurt samples were $\sim 10^8$ CFU/g. *S.thermophilus* was always present in higher numbers than *L.bulgaricus*. Counts of *S.thermophilus* and *L.bulgaricus* were significantly higher ($P < 0.05$) in control than in QSW yogurts (Fig. 3b), which is correlated with the lower pH of control yogurts. Numbers of *L.bulgaricus* decreased faster than did those of *S.thermophilus* which remained stable throughout the storage period. Previous studies have reported that the most important contributing factors for loss of cell viability are decreasing pH during product storage (post-acidification) and the accumulation of organic acids as a result of growth and fermentation (Kailasapathy, Harmstorf, & Phillips, 2008). Furthermore, QSW was already acidic and rich in organic acids although pH was not affected due to the buffering capacity of milk.

The presence of high amounts of formic acid and acetic acid may have accounted for the inhibitory effect of QSW on lactobacilli. In fact, Fattouch et al. (2008) reported antibacterial effect of quince against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Reported minimum inhibitory concentrations were quite low, from 10^2 to 10^4 μg polyphenols/mL.

Certain yeasts play an important role in the spoilage of fermented products. Since milk is pasteurized before yogurt production, the presence of yeasts in yogurt is caused by recontamination processes during manufacture, and can be a problem in fruit-containing yogurts (Nogueira et al., 1998). Numbers of yeasts and moulds were significantly higher in QSW yogurts than in control yogurts ($P < 0.05$) and increased during cold storage, such increase may have been due to recontamination, however we recommend to pasteurize QSW prior to mix it with skim milk powder in order to enhance the microbial quality of the mix. The highest population was 5.00×10^2 CFU/g at day 28 in QSW yogurts.

3.2.6. Evolution of organic acids and sugar content in yogurts during cold storage

Evolution of organic acids and sugars of yogurts are presented in Tables 4 and 5 respectively. Organic acids are important indicators of bacterial metabolic activity in yogurt and they also contribute to the taste and flavor of the product along with other volatile and semi-volatile compounds such as diacetyl and acetaldehyde (Adhikari, Grün, Mustapha, & Fernando, 2002). All organic acids

Table 3

Evolution of rheological parameters of set yogurts enriched with quince scalding water under oscillatory testing (frequency sweep values obtained at 0.1 Hz, 0.4 Pa, 7 °C): G', elastic moduli; G'', viscous moduli; η^*, complex viscosity; tan (δ) and γ deformation during 28 days of cold storage.

Type of yogurt	Storage time (days)	G' (Pa)		G'' (Pa)		η^* (Pa s)		tan δ (−)		γ (−)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	1	721.05^a	39.75	162.70^a	7.20	1175.00^a	65.00	0.226^b	0.003	0.00054	0.00003
	7	753.70^a	153.71	163.20^a	35.21	1229.00^a	250.99	0.230^b	0.010	0.00057	0.00008
	14	1264.47^b	309.76	294.90^b	84.55	2066.67^b	511.25	0.215^a	0.005	0.00034	0.00013
	21	1160.33^b	216.71	245.27^b	44.49	1886.67^b	352.44	0.212^a	0.001	0.00036	0.00006
	28	1179.00^b	87.88	247.93^b	17.29	1916.67^b	141.11	0.210^a	0.003	0.00034	0.00026
Quince	1	417.80^a	43.18	102.70^a	7.89	685.00^a	63.24	0.246^b	0.002	0.00093^b	0.00001
	7	705.73^b	69.09	173.17^b	18.74	1156.67^b	112.60	0.245^b	0.005	0.00056^a	0.00005
	14	790.00^b	13.60	188.40^b	4.40	1295.00^b	25.00	0.239^{a,b}	0.002	0.00049^a	0.00001
	21	789.97^b	116.36	183.50^b	26.93	1293.00^b	191.29	0.232^a	0.002	0.00052^a	0.00008
	28	640.55^b	28.95	150.80^c	4.10	1045.00^b	45.00	0.236^a	0.005	0.00061^a	0.00003

S.E., standard error.
Values with different superscript letters within the same sample and column significantly differ (Tukey's test, $^*P < 0.05$).

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Table 4
Evolution of oxalic, citric, tartaric, lactic, acetic, malic, ascorbic and fumaric acids (mg/100 g) of yogurts enriched with quince scalding water during 28 days of cold storage.

Organic acid (mg/100g)	Control yogurt					Quince scalding water yogurt				
	1	7	14	21	28	1	7	14	21	28
Oxalic acid	1.49^{b} (0.08)^{d}	1.49^{b} (0.06)	1.23^{a,b} (0.09)	1.09^{a} (0.10)	1.14^{a} (0.03)	3.54 (0.11)	3.11 (0.70)	1.62 (0.53)	1.57 (0.71)	2.82 (0.56)
Citric acid	198.94 (13.02)	183.21 (6.02)	177.96 (10.89)	171.21 (11.07)	187.65 (9.79)	257.82 (1.08)	274.69 (46.80)	180.73 (7.38)	182.52 (21.81)	249.17 (32.79)
Tartaric acid	265.5 (35.06)	221.82 (9.84)	225.58 (20.60)	229.4 (25.19)	244.48 (21.46)	303.5 (21.54)	363.32 (43.57)	259.52 (13.96)	249.64 (22.42)	335.03 (40.24)
Lactic acid	1192.56 (55.72)	1333.3 (54.73)	1294.17 (107.65)	1306.54 (98.79)	1383.85 (54.59)	1364.51 (89.65)	1612.28 (289.75)	1196.45 (24.01)	1318.41 (156.18)	1648.53 (179.96)
Acetic acid	11.13^{a} (0.39)	12.75^{a} (0.60)	13.33^{a,b} (0.88)	15.19^{b,c} (0.80)	16.01^{c} (1.12)	50.53 (4.95)	63.11 (12.53)	42.67 (1.21)	49.74 (6.25)	65.01 (6.76)
Malic acid	N.d.	N.d.	N.d.	N.d.	N.d.	7.49 (0.64)	4.96 (1.35)	9.15 (2.26)	5.69 (0.08)	8.87 (3.05)
Ascorbic acid	N.d.	N.d.	N.d.	N.d.	N.d.	0.84 (0.07)	0.88 (0.18)	0.78 (0.01)	0.69 (0.12)	1.33 (0.20)
Fumaric acid	N.d.	N.d.	N.d.	N.d.	N.d.	4.60^{c} (0.52)	2.33^{a,b} (0.47)	3.12^{b,c} (0.19)	1.07^{a} (0.06)	2.84^{a,b,c} (0.28)
TOTAL ACIDITY (g/100 g)	1.67 (0.14)	1.76 (0.02)	1.71 (0.21)	1.73 (0.19)	1.84 (0.17)	1.99 (0.07)	2.32 (0.40)	1.69 (0.05)	1.81 (0.21)	2.31 (0.26)

N.d. = non detected.
Values with different superscript letters within the same line significantly differ (Tukey's test, *$P < 0.05$). No letters when no differences have been detected.
[d] Values in parentheses denote standard error.

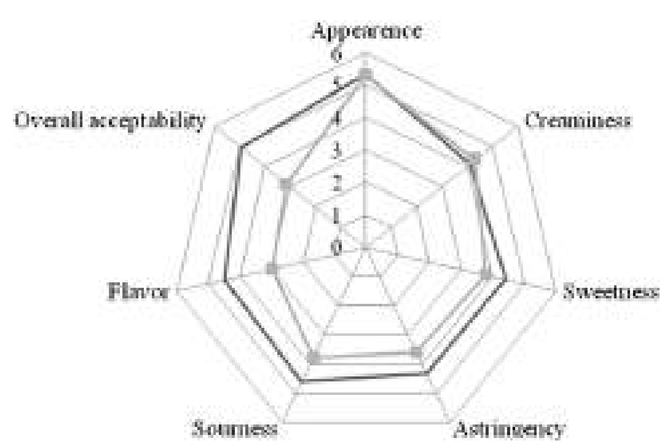

Fig. 2. Scores obtained by sensory evaluation of of control yogurts (▬) and quince enriched yogurts (▬■▬) (7 point hedonic scale). Data are the average ($n = 36$) (Tukey's test, *$P < 0.05$).

detected were significantly ($P < 0.05$) affected by the type of yogurt (Table 4). QSW yogurts had slightly higher concentration of oxalic acid ($P < 0.05$) than control yogurts. Citric and tartaric acids were also present at similar concentrations but slightly lower ($P < 0.05$) in control yogurts. Lactic acid was the major organic acid. Acetic acid was detected at greater levels in QSW yogurts due to its highest content in the raw material. Also, acetic acid can be produced from citrate, lactose and amino acids (Ong & Shah, 2009). Malic, fumaric and ascorbic acids were only detected in QSW yogurts. No significant differences in the content of organic acids

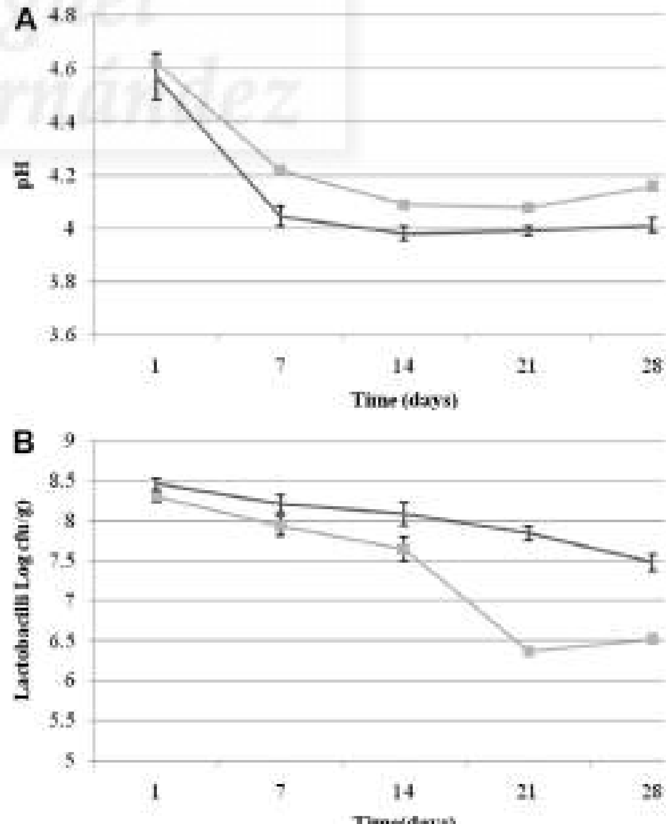

Fig. 3. Evolution of pH (A), and counts of lactobacilli (B) of control yogurts (▬) and quince enriched yogurts (▬■▬) stored at 4 °C for 28 days. Data are the average ($n = 9$) ± standard error (Tukey's test, *$P < 0.05$).

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Table 5
Evolution of maltohexaose and glucose (mg/100 g), galactose and lactose (g/100 g) of yogurts enriched with quince scalding water during 28 days of cold storage.

Type of yogurt	Storage time (days)	Maltohexaose (mg/100 g)		Glucose (mg/100 g)		Galactose + Fructose (g/100 g)		Lactose (g/100 g)		*TOTAL SUGARS* (g/100 g)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	1	84.66	3.43	N.d.	–	1.12	0.06	4.35	0.12	5.55	0.07
	7	76.61	1.82	N.d.	–	1.24	0.03	4.17	0.18	5.49	0.24
	14	72.67	7.12	N.d.	–	1.20	0.10	3.76	0.24	5.03	0.13
	21	72.21	7.26	N.d.	–	1.21	0.09	3.68	0.20	4.97	0.25
	28	83.07	2.48	N.d.	–	1.29	0.07	4.04	0.11	5.41	0.18
Quince	1	100.76	0.00	411.24	13.70	2.34	0.11	6.30	0.24	9.15	0.37
	7	102.77	19.24	416.94	84.25	2.58	0.52	6.29	1.42	9.38	2.05
	14	72.82	3.87	296.12	5.26	1.85	0.04	4.31	0.10	6.53	0.13
	21	72.36	12.96	291.93	29.64	1.95	0.22	4.27	0.50	6.58	0.77
	28	92.64	12.70	406.44	48.62	2.54	0.31	5.77	0.80	8.81	1.17

N.d. = non detected.
S.E., standard error.

were detected in yogurts during storage time except for lactic acid which increased with storage time ($P < 0.05$).

Lactose decreased with storage time (Table 5). Galactose increased or remained constant in all yogurts ($P > 0.05$) which agreed with results reported by Wang et al. (2010). This is because of the cultures used in yogurt fermentation (*L. bulgaricus* and *S. thermophilus*) utilize the glucose moiety of lactose, but not the galactose moiety. Thus, while lactose and glucose content in fermented product decrease, the galactose content remains unchanged (O'Brien, 1999). Lactose content is much lower ($P < 0.05$) in control yogurts than in QSW yogurts, which is correlated with the lower lactobacilli counts in QSW yogurts. Glucose was detected in QSW yogurts as it is provided by QSW and ranged from 0.3 to 0.4 g/100 g in yogurts.

4. Conclusions

Hot water extracts from different fruits are a good source of different compounds such as polysaccharides, sucrose, minerals, phenols and flavonoids, and many of them have antioxidant activity. Quince scalding water is rich in phenolic compounds and flavonoids which provide interesting antioxidant properties, and also contain organic acids and sugars that are all extracted during scalding. The addition of quince scalding waters provides color changes and reduced the sensory scores of yogurts due to its acidic nature. Such scalding water has inhibitory effect against lactobacilli, probably due to its high content in polyphenols. As a consequence, quince scalding water enriched yogurts have higher pH, lower lactic acid content and probably affected microbial metabolism for example reducing the release of exopolysaccharides from lactic acid bacteria compared to control yogurts. Such changes are reflected in their rheological and textural properties: softer yogurts of higher deformability and lower elastic behavior and viscosity. During cold storage of yogurts pH decreases, the gel structure is reinforced, lactobacilli population decreases (especially in quince enriched yogurts) and population of molds and yeasts increases. The direct use of heat treated QSW, although nowadays limited by regulations may enhance the eco-efficiency of quince industries. Further researches will be needed as well, to study the possibility of recuperate bioactive compounds from QSW that would allow their use as food ingredients (for their antibacterial or antioxidant properties) or as health promoting agents to ameliorate illness (such as allergy) or improve the nutritional value of conventional foods.

References

Adhikari, K., Grün, I. U., Mustapha, A., & Fernando, L. N. (2002). Changes in the profile of organic acids in plain ser and stirred yogurts during manufacture and refrigerated storage. *Journal of Food Quality, 25*, 435–451.

Ares, G., Paroli, C., & Harte, F. (2006). Measurement of firmness of stirred yogurt in routine quality control. *Journal of Food Quality, 29*, 628–642.

Augustin, M. A., Clarke, P. T., & Craven, H. (2003). Powdered milk: characteristics of milk powders. In B. Caballero (Ed.), *Encyclopaedia of food science and nutrition* (2nd ed). (pp. 4703–4711). London: Elsevier Science.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, P. M., Albertini, M. C., & Piatti, E. (2005). Raw Millefiori honey is packed full of antioxidants. *Food Chemistry, 97*, 217–222.

Casani, S., Rouhany, M., & Knøchel, S. (2005). A discussion paper on challenges and limitations to wáter reuse and higiene in the food industry. *Water Research, 39*, 1134–1146.

Dave, R. I., & Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal, 7*, 31–41.

Doughty, E. (1995). Separation of acids, carbohydrates and feremention products by HPLC. *Lebensmittel & Biotechnologie, 12*(3), 100–101.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessi, S., et al. (2008). Comparative analysis of polyphenols profiles and antioxidant and antimicrobial activities of Tunisian pome fruit pulp and peel aqueous acetone extracts. *Journal of Agricultural and Food Chemistry, 56*(3), 1084–1090.

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García, E., Gozálvez, J. M., & Lora, J. (2002). Use of reverse osmosis as a preconcentration system of waste leaching liquid from the citric juice production industry. *Desalination, 148*, 137–142.

Heller, K. J. (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. *The American Journal of Clinical Nutrition, 73*, 374S–379S.

Hsu, B., Coupar, I. M., & Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. *Food Chemistry, 98*(2), 317–328.

ISO. (1988). *International Standard 8589. Sensory analysis general guidance for the design of test rooms*. Ref. No. ISO 8589:1988 (E). Geneva: International Organization for Standardization.

Jiménez, A. M., Murcia, M. A., Parras, P., & Martínez-Tomé, M. (2008). On the importance of adequately choosing the ingredients of yoghurt and enriched milk for their antioxidant activity. *International Journal of Food Science and Technology, 43*, 1464–1473.

Kailasapathy, K., Harmstorf, I., & Phillips, M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp *lactis* in stirred fruit yogurts. *LWT-Food Science and Technology, 41*, 1317–1322.

Lubbers, S., Decourcelle, N., Vallet, N., & Guichard, E. (2004). Flavor release and rheology behaviour of strawberry fat-free stirred yogurt during storage. *Journal of Agricultural and Food Chemistry, 52*(10), 3077–3082.

Magalhaes, A. S., Silva, B. M., Pereira, J. A., Andrade, P. B., Valentao, P., & Carvalho, M. (2009). Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes. *Food and Chemical Toxicology, 47*, 1372–1377.

Mandal, S., Sarkar, R., Patra, P., Nandan, C. K., Das, D., Bhanja, S. K., et al. (2009). Structural studies of a heteropolysaccharide (PS-I) isolated from hot water extract of fruits of *Psidium guajava* (Guava). *Carbohydrate Research, 344*(11), 1365–1370.

Nogueira, C., Albano, H., Gibbs, P., & Teixeira, P. (1998). Microbiological quality of Portuguese yogurts. *Journal of Industrial Microbiology & Biotechnology, 21*, 19–21.

O'Brien, J. (1999). Sugar profiles of cultured dairy products in the UK. *Journal of Human Nutrition and Dietetics, 12*, 245–250.

Ong, L., & Shah, N. P. (2009). Probiotic cheddar cheese: influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. *LWT-Food Science and Technology, 42*, 1260–1268.

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., et al. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry, 97*, 137–150.

Robinson, R. K. (2003). Yoghurt, role of starter cultures. In H. Roginski (Ed.), *Encyclopedia of dairy sciences* (pp. 1059–1063). London, UK: Elsevier Science Ltd.

Rodríguez-Guisado, I., Hernández, F., Melgarejo, P., Legua, P., Martínez, R., & Martínez, J. J. (2009). Chemical, morphological and organoleptical characterisation of five Spanish quince tree clones (*Cydonia oblonga* Miller). *Scientia Horticulturae, 122*, 491–496.

Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C., & Pérez-Álvarez, J. A. (2010). Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. *LWT-Food Science and Technology, 30*, 1–7.

Shinomiya, F., Hamauzu, Y., & Kawahara, T. (2009). Anti-allergic effect of a hot-water extract of quince (*Cydonia oblonga*). *Bioscience, Biotechnology and Biochemistry, 78*(8), 1773–1778.

Silva, B. M., Andrade, P. B., Ferreres, F., Domingues, A. L., Seabra, R. M., & Ferreira, M. A. (2002). Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *Journal of Agricultural and Food Chemistry, 50*, 4615–4618.

Silva, B. M., Andrade, P. B., Valentao, P., Ferreres, F., Seabra, R. M., & Ferreira, M. A. (2004). Quince (*Cydonia oblonga* Miller) fruit (pulp, peel and seed) and jam: antioxidant activity. *Journal of Agricultural and Food Chemistry, 52*, 4705–4712.

Silva, B. M., Andrade, P. B., Martins, R. C., Valentao, P., Ferreres, F., Seabra, R. M., et al. (2005). Quince (*Cydonia oblonga* Miller) fruit characterization using principal component analysis. *Journal of Agricultural and Food Chemistry, 53*, 111–122.

Singh, N., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry, 85*, 611–616.

Steffe, J. F. (1996). *Rheological methods in food process engineering*. East Lansing, Michigan: Freeman Press.

Singh, G., & Muthukumarappan, K. (2008). Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. *LWT-Food Science and Technology, 41*, 1145–1152.

Viuda-Martos, M., Ruiz Navajas, Y., Sánchez Zapata, E., Fernández-López, J., & Pérez-Álvarez, J. A. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *Flavour and Fragance Journal, 25*, 13–19.

Yang, X.-M., Yu, W., Ou, Z.-P., Ma, H.-L., Liu, W.-M., & Ji, X.-L. (2009). Antioxidant and immunity activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. *Plant Foods for Human Nutrition, 64*(2), 167–173.

Wang, J., Guo, Z., Zhang, Q., Yan, L., Chen, Y., Chen, X., et al. (2010). Effect of probiotic *Lactobacillus casei* Zhang on fermentation characteristics of set yogurt. *International Journal of Dairy Technology, 63*(1), 105–112.

PUBLICACIÓN 2

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Nutritional and Antioxidant Properties of Date Pastes and Blanching Water Obtained from by-Products of Medjoul and Confitera Cultivars

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Abstract The date industry faces economic losses due to non-conformities with quality standards (pre and post-harvest defects). The present study deals with two types of discarded date palm: Medjoul (post-harvest defects) and Confitera (pre-harvest cracks) with the aim to obtain valuable products. Dates were processed to obtain paste and blanching water. Nutritional value and antioxidant properties of the paste and blanching water were determined. Medjoul paste and blanching water have demonstrated to be a good source of sugars (33.02% and 5.44%, respectively) whereas Confitera paste and blanching water have an important content in phenols and flavonoids, which in particular confers antioxidant properties to Confitera blanching water. Furthermore both date pastes are a good source of fiber. Blanching process especially affects Confitera cultivar since a huge amount of organic acids, phenolics and flavonoids are solubilized and leaked during date processing, increasing the bioactive content in Confitera blanching water. These results showed the opportunity to incorporate these new products from non-commercial dates as functional ingredients into the food chain helping, whereas improving, waste management in the date industry.

Keywords Date Palm, Date Paste, Blanching Water, Waste Management, Valorization

1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important cash crops; it is especially grown in Arab region and the area bordering the Mediterranean coast [1]. The economic importance of date palm is due to its nutritionally valuable fruit, which consists of 44-88% sugar, fat (0.2-0.5%), minerals such as potassium (2.5 times more than bananas), calcium, magnesium and iron, protein (2.3-5.6%), dietary fiber (6.4-11.5%) as well as vitamins and amino acids [2]. Moreover Baliga[3] recently performed a comprehensive review of the biological and pharmacological activities of date fruits. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflamatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities [3].

The annual world date production in 2011 was 7,302,703 tonnes of which 15,327 tonnes were produced in Europe, an increase of 2.71% since 2010 [4]. However, a significant portion of dates is wasted in date producing countries (2,000,000 per year globally) due to its inferior quality, damage, and undersized fruit or unattractive appearance [5]. It is also reported that dates are also wasted during sorting, storage and conditioning. The non-use of lesser-quality dates constitutes a real economic loss since such dates are still rich in bioactive compounds which can be extracted and used as valuable ingredients [6].

Tonnes of wastes are discarded daily by the date processing industries leading to environmental problems. Date processing industries manufacture a variety of date products such as date-paste, date-syrup, date dip, date-honey, date-jam and date-vinegar. The dates are generally steamed, destoned, macerated, and converted to a semi-solid form known as paste [7,8]. Nowadays there is a growing interest in the reuse and valorization of water from the food industry and the water used for steam dates during its processing could contain an important amount of bioactive compounds presents in the date fruit. Overcome the barrier of legislation and hygienic concerns through selecting an appropriate treatment system to ensure microbiological and chemical quality [9,10] would generate new food applications for scalding water from date industries.

The aim of this study was to evaluate the nutritional quality and antioxidant properties of the paste and blanching water obtained from two date cultivars discarded during the industrialization, for its valorization as ingredients in fortified foods.

2. Materials and Methods

2.1. Plant Material

This study was conducted on non-commercial Medjoul and Confitera date fruits procured from 'Estación Phoenix' (Elche, Alicante, Spain): Medjoul dates rejected after dates selection due to low post-harvest quality (broken, uneven size or color, with unattractive appearance and/or undersized); and prematurely harvested Confitera dates. Confitera is a variety that needs high temperatures during maturation, and Elche has the palm grove located at the northern latitude, so Elche's climatic conditions are not optimal, and commonly such dates have to be collected unripened due to fruit cracks. Dates are ripen in four stages known throughout the world by their Arabic names: "kimri" (unripe; moisture content of 85 %), "khalal" (full-size, crunchy; 50-60 % moisture), "rutab" (ripe, soft; 35-40 % moisture) and "tamr" (ripe, reduced moisture about 20 %) [11]. Medjoul dates were at the "tamr" stage and Confitera dates were at "rutab" stage. Those are the ripening stages were most discarded dates are generated, for each variety. Only dates to be discarded were used.

2.2. Date Paste and Blanching Water Preparation

Date fruits were washed under running water at 40 °C to remove dust and macroscopic contamination, and afterwards scalded at 100 °C (1:1 water/fruit) for three minutes. After that, the seeds and the peels were manually removed and the flesh was crushed to obtain a homogeneous paste. This paste was vacuum-packed and kept frozen at -30 °C till analysis. Blanching water from dates was filtered through cotton gauzes to remove particles, pasteurized in a bath at 80 °C during 30 min, cooled and frozen at -30 °C till analysis.

2.3. Physicochemical analysis

2.3.1. Measurement of pH and °Brix

The pH was measured using a pH-meter (model pH/Ion 510, Eutech Instruments Pte Ltd., Singapore). The total soluble solids (TSS), expressed as °Brix, were measured using a refractometer (Mod. DR-101, Coseta S.A., Barcelona, Spain). Both measurements were taken at 20 °C.

2.3.2. Proximate composition

Ash, protein and fat content were determined according to Official Methods [12]. Moisture content was measured by loss in weight after heating a 3 g sample to constant weight at 60-65 °C in a vacuum oven. Total (TDF) and insoluble dietary fiber (IDF) were determined following the enzymatic-gravimetric AOAC method 991.43 using MES-TRIS buffer. Soluble dietary fiber (SDF) was calculated by subtracting the IDF proportion from TDF. These results were presented as percentage in the dry matter (DM).

2.3.3. Sugars and organic acids analysis

Contents of organic acids and sugars in date pastes and blanching water were analyzed. Samples (5g) were homogenized in 10 mL ultrapure water acidified with 0.1 % ortophosphoric acid and shaken vigorously (IKA® T25 digital ULTRA-TURRAX®, IKA® Werke Staufen, Germany) for 20 s at 13,500 rpm and centrifuged for 20 min at 15,000 rpm at 4 °C. The supernatants fluids were filtered through 0.45 μm membrane filters (Millipore Corporation, Bedford, USA). Samples (10 μL) were injected in a cation exchange column (Supelcogel C-610 H, 300 x 7.8 mm, Supelco, Bellefonte) with a pre-column (Supelguard-H, 50 x 4.6 mm, Supelco, Bellefonte), using 0.1 % H_3PO_4 as mobile phase, at an operating flow rate of 0.5 mL/min. A Hewlett Packard HP-1100 instrument (Woldbronn, Germany) coupled with two detectors: DAD G1315A (set at 210 nm) and RID G-1362A was used. Standards of organic acids, monosaccharides and oligosaccharides were obtained from Supelco. Samples were run at 30 °C and the run time was 30 min [13]. Peaks were identified by comparison with retention time of the standards, and quantified by regression formula obtained with the standards. Results were expressed in fresh weight basis (FW).

2.3.4. Measurement of total phenol content and total flavonoid content

Reagents and methods run were according to Viuda-Martos[14].

The total phenol content (TPC) was determined using Folin-Ciocalteu's reagent [15] and results were expressed as mg gallic acid equivalents (GAE)/g sample. For total flavonoid content (TFC), the method based on Blasa[16] was used and results were expressed as mg rutin equivalents (RE)/100 g of sample. Results were referred to fresh weight basis (FW).

2.4. Antioxidant properties

Antioxidant activity was measured by three methods: i) The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined following the method of Viuda-Martos[14]; the amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50% was calculated graphically, ii) The ferric reducing power (FRAP) was determined by using the potassium ferricyanide-ferric chloride method [17] and estimated in terms of Trolox equivalent antioxidant capacity (TEAC) in mmol/L Trolox; iii) The ferrous ion-chelating (FIC) assay was carried out according to the method of Singh[18]; to determine the concentration needed to obtain 50% chelating effect (EC_{50}), the percentage of chelating effect was plotted against sample concentration.

2.5. Statistical analysis

Data was analyzed by one-way ANOVA test (SPSS

statistical software, version 20.0, Chicago, IL, USA), using Tukey's pairwise comparisons post-hoc test with a significance level of 0.05. Three independent batches of 2 kg of dates were processed for each date cultivar. All determinations were run in triplicate, except fiber determinations that were duplicated.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Proximate composition

Table 1 presents the average composition of date pastes and blanching water. Regarding pH values, MP and MBW presented a pH close to neutrality making them suitable for incorporating into many foods. CP and CBW with a lower pH could modify food acidity when added, which needs to be taken into account for potential uses. Date paste has a great tendency to retain water [19] and so CP presented very high moisture. Also, MP presented high moisture, surely increased compared to data from fresh "tamr" Medjoul fruit (34.73 ± 1.16 g/100 g). Furthermore, it was demonstrated that blanched date fruits captured a plus of water [19]. Results for pH and moisture were in accordance to those obtained from Martín-Sánchez[20] in a similar Confitera date paste.

Date pastes were characterized by a high sugar content, and MP had the highest total soluble solids (TSS) values ($p<0.05$) as a consequence of fruit maturity. MP presented higher content of protein, ash, TDF, IDF and SDF ($p<0.05$) than CP. TDF in dried dates has been found between 8.1-12.3% dry matter (DM) [21]. Thus, both date pastes analyzed in the present study could be considered high in TDF. It is interesting to note that Martín-Sánchez[19] reported that blanching improves some technological properties of dates. In blanched dates the water holding capacity and the emulsion stability are increased, which are very important properties to obtain a desirable texture in some products [19]. On the other hand MBW showed higher TSS, protein and ash ($p<0.05$) than CBW. The relatively high TSS content of the MBW makes it an interesting source of sugars.

3.1.2. Sugars

Sugars are the major chemical constituent of date pastes being this content higher ($p<0.05$) in MP (33.02%) than in CP (22.53%) due to variety and maturation stage, indeed total sugar contents in dates increase from the early stage (Kimri) to the last stage of maturity (Tamr). Date pastes were mainly composed by glucose and fructose; these two types of reducing sugars have approximately equal concentration in both cultivars (Figure 1) as the ratio Glu/Fru informed. CBW had the lowest sugar content (1.28%) and sucrose was the predominant sugar, representing nearly the 75% of the total sugar content. MBW contained about 15 times more glucose and fructose than CBW, as reported in previous studies [10], which is explained by the stage of ripening of date fruits.

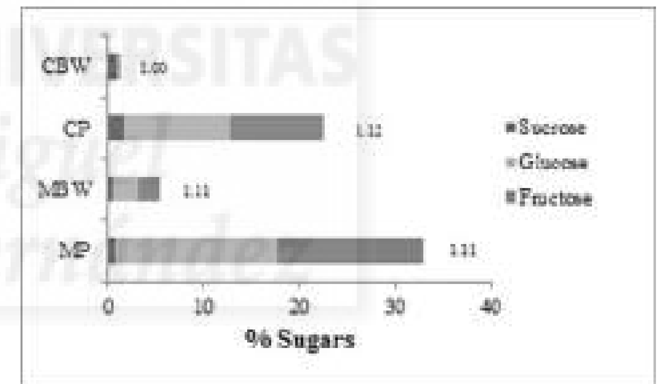

Figure 1. Sugar profile of the date pastes and blanching water (g/100 g, mean values). The numbers at the end of each bar represent the ratio Glu/Fru

Table 1. pH, total soluble solids (ºBrix), moisture, protein, fat, ash, total dietary fibre (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) of Medjoul and Confitera date pastes and blanching water (mean values ± error standard)

	MP	MBW	CP	CBW
pH	6.98 ± 0.01^b	6.25 ± 0.03^B	5.80 ± 0.00^a	5.70 ± 0.00^A
ºBrix	39.8 ± 0.58^b	8.00 ± 0.10^B	29.73 ± 0.07^a	2.17 ± 0.05^A
Moisture (g/100 g)	60.2 ± 0.47^a	93.86 ± 0.12^A	70.27 ± 0.27^b	97.97 ± 0.17^B
Protein* (g/100 g)	3.51 ± 0.10^b	0.71 ± 0.09^B	2.62 ± 0.11^a	0.19 ± 0.07^A
Fat* (g/100 g)	0.17 ± 0.04^b	---	0.13 ± 0.05^a	---
Ash* (g/100 g)	3.32 ± 0.12^b	0.67 ± 0.07^B	2.48 ± 0.10^a	0.18 ± 0.02^A
TDF* (g/100 g)	12.43 ± 0.18^b	n.d.	9.29 ± 0.08^a	n.d.
IDF* (g/100 g)	10.22 ± 0.46^b	n.d.	7.63 ± 0.22^a	n.d.
SDF* (g/100 g)	2.21 ± 0.14^b	n.d.	1.64 ± 0.05^a	n.d.

*Values are given on dry matter (DM).

Abbreviations: MP, Medjoul paste; MBW, Medjoul blanching water; CP, Confitera paste; CBW, Confitera blanching water; n.d.: not determined.

Values with different small superscript letters within the same line significantly differ ($p<0.05$) between pastes.

Values with different capital superscript letters within the same line significantly differ ($p<0.05$) between blanching water.

Table 2. Organic acid (mg/100 g) profile of date pastes and blanching water (mean values ± error standard)

	MP	MBW	CP	CBW
Oxalic acid	53.29 ± 2.51^{b}	0.22 ± 0.03^{A}	41.41 ± 2.94^{a}	4.29 ± 0.11^{B}
Citric acid	155.43 ± 3.97	9.72 ± 0.83^{A}	159.93 ± 12.46	70.27 ± 2.06^{B}
Malic acid	764.58 ± 0.38^{b}	15.78 ± 0.76^{A}	579.01 ± 43.07^{a}	22.22 ± 0.21^{B}
Ascorbic acid	12.64 ± 1.54	N.d.	11.14 ± 1.09	N.d.
Succinic acid	507.79 ± 9.19^{a}	142.28 ± 21.61^{A}	640.19 ± 53.07^{b}	276.00 ± 17.20^{B}
Formic acid	111.68 ± 21.55	5.71 ± 0.18^{A}	129.31 ± 19.83	52.40 ± 2.31^{B}
Acetic acid	179.15 ± 25.54	33.50 ± 5.01^{A}	244.08 ± 37.14	198.74 ± 1.76^{B}
Fumaric acid	187.45 ± 0.33^{b}	9.18 ±1.32^{A}	61.33 ± 5.85^{a}	52.44 ± 3.07^{B}
TOTAL ACIDITY (%)	1.97 ± 0.05	0.22 ± 0.03^{A}	1.87 ± 0.18	0.68 ± 0.04^{B}

Abbreviations: MP, Medjoul paste; MBW, Medjoul blanching water; CP, Confitera paste; CBW, Confitera blanching water; N. d., not detected.
Values with different small superscript letters within the same line significantly differ ($p<0.05$) between pastes.
Values with different capital superscript letters within the same line significantly differ ($p<0.05$) between blanching water

3.1.3. Organic acids

Table 2 shows the organic acid profile of date pastes and blanching water. In MP malic acid was the predominant organic acid, as other authors pointed out [22,23]. From a technological point of view being malic acid the predominant acid it is a positive trait since it acts as a flavour enhancer [19]. In CP, CBW and MBW succinic acid was the major organic acid, succinic is also a tasty organic acid. In pastes other organic acids were presents at lower concentrations such as acetic, fumaric and citric acids. Ascorbic acid was only present in pastes, may be due to thermal inactivation of this acid during blanching. The inner parts of the fruit (origin of pastes) retained some ascorbic acid content whereas the blanching water reached boiling temperatures for longer times and so ascorbic was completely inactivated. In CBW a relative high concentration of formic acid was found, which has previously demonstrated to stimulate the growth of lactobacilli [24]. Both pastes had similar total acidity ($p>0.05$) but among blanching water CBW had higher total acidity content ($p<0.05$) than MBW, so the blanching process highly affects Confitera dates as a largeramount of the organic acids are solubilized and leaked into the water.

3.1.4. TPC and TFC

Factors such as variety, growing condition and maturity among others, might be responsible for the observed differences in TPC and TFC values (Figure 2) [25]. Indeed, natural drying occurred during dates ripening is regarded as unfavorable for antioxidants, due to the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds [26]. Confitera was the date variety which presented the highest TPC and TFC ($p<0.05$), both in paste and blanching water (Figure 2), although such phenolic content could reach values of 14.500 mg GAE/L for the same cultivar in the same maturation stage [20]. Besides, CBW appears as a source of phenolics as its content is similar to that of date pastes. Different results have been found by other authors in date fruits. Amorós[22] reported values up to 4000 mg of GAE/L in the early Khalal maturation stage whereas Mansouri[27] found that TPC ranged from 24.9 to 83.6 mg of GAE/L in ripe date fruits.

Flavonoids possess diverse health benefits, which include antioxidant and radical scavenging activities, among others [3]. However, the composition of flavonoids and other antioxidant phenolics in fruit changes dramatically during ripening [28]. The highest TFC was found in CP, followed by CBW. Chaira[29] reported the highest content of flavonoids in the Korkobbi variety (54.46 quercetin equivalents/100 g) which supposedly was responsible for the highest antiradical efficiency of this cultivar.

In relation to individual phenolic compounds significant differences were found in the literature regarding date cultivar and origin, as well as the maturation stage in which date fruits were found. Normally hydroxycinnamic acids (HCAs), including chlorogenic, caffeic, ferulic, *p*-, *m*-, *o*-coumaric and cinnamic acids are the most predominant group of phenolic acids. Hydroxybenzoic acids (HBAs),including gallic, protocatechuic, syringic, isovanillic and 3-hydroxybenzoic acids are found at lesser amounts [27,30,31]. However, other authors found higher levels of HBAs in their date fruit samples [32]. Also, Amira et al.[31] identified and quantified two other phenolic acids, hydroxyphenylacetic and phenylacetic acids. Regarding the ripening stage, HBAs are found with an appreciate amount until maturation of date fruit [31]. On this basis Confitera dates are expected to present higher amounts of HBAs than Medjoul dates. Flavonoids namely catechin, apinegin, isoquercetin, rutin, quercetrin, quercetin and luteolin were identified in the literature [30,31,32]. Furthermore Hong et al[33] identified several procyanidin oligomers and flavonoid glycosides of lutein, quercetin and apinegin in Deglet Nour cultivar. The beneficial effects derived from individual phenolic compounds have been attributed to their antioxidant activity [34] and several studies have proven the contribution of dietary polyphenols to human health [35,36,37].

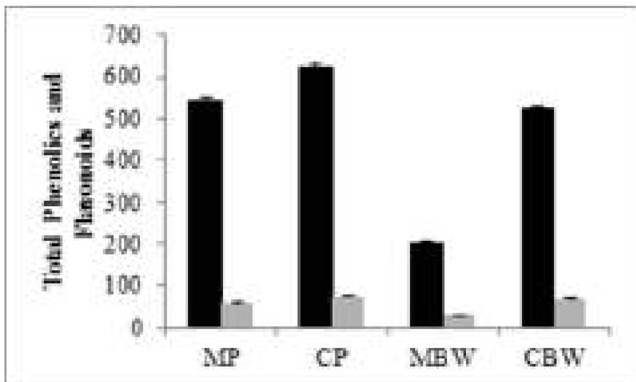

Figure 2. Total phenol content (TPC; mg GAE/L) and total flavonoid content (TFC; mg RE/100 g) of Medjoul and Confitera pastes (MP, CP) and blanching water (MBW, CBW) (■ TPC, ■ TFC).

3.2. Antioxidant Properties

Due to the antioxidant activity (AA) measured by an individual assay reflects only the chemical reactivity under the specific conditions applied in that assay a mixture of methods should be used when assessing AA *in vitro* to cover all the aspects of antioxidant efficacy [38]. Three assays based on spectrophotometry methods of antioxidant capacity were used in this study.

Regarding the DPPH assay (Table 3) CBW showed the highest ($p<0.05$) ability to inhibit DPPH radical at 10 g/100 g. The DPPH scavenging data suggest that the extract is capable of scavenging free radicals, hence preventing the initiation and propagation of free-radical-mediated chain reactions [39]. The higher antioxidant activity, the lower IC_{50} value; in this sense the order of AA was: CP<MBW<CBW<MP.

Analysis of the reducing properties showed (Table 4) that pastes had a low antioxidant action. CBW showed the highest values ($p<0.05$) of TEAC over all samples analyzed and did so in a concentration-dependent manner.

Table 3. Antioxidant activity of date pastes and blanching water at different concentrations (A = 2.5 %, B = 5 %, C = 7.5 %, D = 10 %), measured by the DPPH method.

Type of material	DPPH Inhibition (%)				
	A	B	C	D	$^1IC_{50}$
Medjoul paste	1.93 (0.15)	1.25^{A} (0.69)	2.24^{A} (0.46)	3.99 (0.99)	34.71
Confitera paste	3.69 (0.39)	3.69^{B} (0.08)	4.22^{B} (0.31)	4.91 (1.15)	69.28
Medjoul BW	5.60^{X} (0.47)	6.71^{X} (0.53)	6.66^{X} (0.16)	7.77^{X} (0.22)	41.51
Confitera BW	9.60^{xY} (0.32)	15.16^{yY} (0.59)	17.37^{zY} (0.16)	17.18^{zY} (0.27)	37.70

[1]IC_{50}, concentration (%) for a 50% inhibition. BW blanching water.

Values with different small superscript letters within the same line significantly differ ($p<0.05$).

Values with different capital superscript letters within the same column significantly differ ($p<0.05$).

*Values in parentheses denote standard error.

Analysis of the metal ion-chelating properties showed (Table 5) that both blanching water were capable of chelating iron (II) in a concentration dependent manner. However, pastes showed no ion-chelating activities at any of the tested concentrations. CBW presented the highest values of metal chelation ($p<0.05$) at all concentrations tested, which is reflected in a lower EC_{50}. Chelating ability is of great interest in the food industry due to the transition of metal ions contributes to lipid oxidation, which is the main source of degradation of food products [14].

Taking into consideration the results obtained from the different methods used, CBW showed the strongest antioxidant capacity. Several studies have pointed to the antioxidant activity of the phenolic and flavonoid compounds in dates [27,40]. Viuda-Martos[41] proved that 4 mL of orange juice waste water showed an equivalent activity to that provided by 0.1 g ascorbic acid or 0.1 g BHT measured by the DPPH method. They also attributed the antioxidant activity of the citrus waste to the phenolic compounds and flavonoids they contained.

Table 4. Antioxidant activity of date pastes and blanching water at different concentrations (A = 2.5 %, B = 5 %, C = 7.5 %, D = 10 %), measured by the FRAP method.

Type of material	FRAP TEAC[1] (mM Trolox/L)			
	A	B	C	D
Medjoul paste	0.02^{aA} (0.00)	0.04^{a,bA} (0.00)	0.05^{b,c} (0.00)	0.07^{c} (0.00)
Confitera paste	0.04^{aB} (0.00)	0.05^{a,bB} (0.00)	0.06^{b} (0.00)	0.08^{c} (0.00)
Medjoul BW	0.09 (0.02)	0.09^{X} (0.00)	0.14^{X} (0.00)	0.22^{X} (0.05)
Confitera BW	0.28^{w} (0.01)	0.47^{xY} (0.03)	0.66^{yY} (0.02)	0.93^{zY} (0.02)

[1]TEAC, Trolox equivalent antioxidant capacity. BW blanching water.

Values with different small superscript letters within the same line significantly differ ($p<0.05$).

Values with different capital superscript letters within the same column significantly differ ($p<0.05$).

*Values in parentheses denote standard error.

In general, BW presented higher antioxidant activities than pastes, that maybe due to the effect of the matrix, much more complex in pastes than in BW. In pastes, the availability or action of substances with antioxidant activity may be hindered. This is also due to the complex mechanisms that lead to the antioxidant activity in which other compounds different from phenolics maybe taking part in the antioxidant mechanism.

Table 5. Antioxidant activity of date pastes and blanching water at different concentrations (A = 2.5 %, B = 5 %, C = 7.5 %, D = 10 %), measured by the FIC method

Type of material	FIC Chelating effect (%)				
	A	B	C	D	[1]EC_{50}
Medjoul paste	N.e	N.e	N.e	N.e	---
Confitera paste	N.e	N.e	N.e	N.e	---
Medjoul BW	1.67^{wX} (0.04)	4.19^{xX} (0.02)	5.12^{yX} (0.05)	11.19^{zX} (0.05)	14.84
Confitera BW	20.49^{wY} (0.12)	30.74^{xY} (0.05)	37.74^{yY} (0.05)	54.26^{zY} (0.06)	9.15

[1]EC_{50}, concentration (%) for a 50% chelating effect. BW blanching water.

Values with different small superscript letters within the same line significantly differ (p<0.05).

Values with different capital superscript letters within the same column significantly differ (p<0.05).

*Values in parentheses denote standard error.

N.e., Not effect.

4. Conclusions

Pastes and blanching water from the date industry could provide an optimal mix of dietary fiber, natural antioxidants and other bioactive compounds to be used as ingredients for food products. Medjoul paste and blanching water have demonstrated to be a good source of sugars, especially reducing sugars, with a nearly neutral pH, that make them suitable to be used by food industries like the bakery, confectionery or dairy industries. Confitera paste and blanching water have an important content in phenols and flavonoids, which in particular confers antioxidant properties to Confitera blanching water, making them good candidates for applications in the meat industry. Therefore, the possibility to successfully incorporate these new functional ingredients from non-commercial dates into the food chain would help the date industry on waste management and valorization.

Blanching process especially affects Confitera cultivar since a huge amount of organic acids, phenolics and flavonoids are solubilized or leaked during date processing. In the scope of the valorization of date fruit by-products and wastes, this fact would made blanching water from Confitera dates an ideal substrate for deriving a range of value added products in food and nutraceutical industries by employing bioprocessing technologies [42].

REFERENCES

[1] J. Aslam, S. H. Khan, S. A. Khan. Quantification of water soluble vitamins in six date palm (Phoenix dactylifera L.) cultivar's fruits growing in Dubai, United Arab Emirates, through high performance liquid chromatography, Journal of Saudi Chemical Society, Vol. 17, 9-16, 2013.

[2] W. Al-Shahib, R. J. Marshall. The fruit of the date palm: Its possible use as the best food for the future?, International Journal of Food Sciences and Nutrition, Vol. 54, No. 4, 247-259, 2003.

[3] M. S. Baliga, B. R. V. Baliga, S. M. Kandathil, H. P. Bhat, P. K. Vayalil. A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.), Food Research International, Vol. 44, 1812-1822, 2011.

[4] FAO. Statistical databases, Online available from http://faostat.fao.org

[5] S. Besbes, L. Drira, C. Blecker, C. Deroanne, H. Attia. Adding value to hard date (Phoenix dactylifera L.): Compositional, functional and sensory characteristics of date jam, Food Chemistry, Vol. 112, 406-411, 2009.

[6] J. Ahmed, A. Almusallam, S. N. Al-Hooti. Isolation and characterization of insoluble date (Phoenix dactylifera L.) fibers, LWT-Food Science and Technology, Vol. 50, 414-419, 2013.

[7] J. Ahmed, H. S. Ramaswamy, A. R. Khan. Effect of water activity on glass transitions of date paste, Journal of Food Enginering, Vol. 66, 253-258, 2005.

[8] A. A. A. Kwaasi. Date palms. Encyclopaedia of Food Science and Nutrition, 2nd ed., Elsevier Science, London, pp. 1730-1740, 2003.

[9] S. Casani, M. Rouhany, S. Knøchel. A discussion paper on challenges and limitations to water reuse and hygiene in the food industry, Water Research, Vol. 39, 1134-1146, 2005.

[10] L. Trigueros, E. Sayas-Barberá, J. A. Pérez-Álvarez, E. Sendra. Use of date (Phoenix dactylifera L.) blanching water for reconstituting milk poder: Yogurt manufacture, Food and Bioproducts Processing, Vol. 90, 506-514, 2012.

[11] F. Biglari, A. F. M. Alkarkhi, A. M. Easa. Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran, Food Chemistry, Vol. 107, 1636-1641, 2008.

[12] AOAC. Official methods of analysis (17th ed. Vol. AOAC), Gaithersburg, MD, 2000.

[13] E. Doughty. Separation of acids, carbohydrates and fermentation products by HPLC, Lebensmittel and Biotechnologie, Vol. 12, No. 3, 100-101, 1995.

[14] M. Viuda-Martos, Y. Ruiz-Navajas, E. Sánchez-Zapata, J. Fernández-López, J. A. Pérez-Álvarez. Antioxidant activity of essential oils of five spice widely used in a Mediterranean diet, Flavour and Fragance Journal, Vol. 25, 13-19, 2010.

[15] V. L. Singlenton, J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enolology and Viticulture, Vol. 16, 144-158, 1965.

[16] M. Blasa, M. Candiracci, A. Accorsi, P. M. Piacentini, M. C.

Albertini, E. Piatti. Raw Millefiori honey is packed full of antioxidants, Food Chemistry, Vol. 97, 217–222, 2005.

[17] M. Oyaizu. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Japanese Journal of Nutrition, Vol. 44, 307-315, 1986.

[18] N. Singh, P. S. Rajini. Free radical scavenging activity of an aqueous extract of potato peel, Food Chemistry, Vol. 85, 611-616, 2004.

[19] A. M. Martín-Sánchez, S. Cherif, J. Vilella-Esplá, J. Ben-Abda, V. Kuri, J. A. Pérez-Álvarez, E. Sayas-Barberá. Characterization of novel intermediate food products from Spanish date palm (Phoenix dactylifera L., cv. Confitera) co-products for industrial use, Food Chemistry, http://dx.doi.org/http://dx.doi.org/10.1016/j.foodchem.2013.12.042

[20] A. M. Martín-Sánchez, G. Ciro-Gómez, E. Sayas, J. Vilella-Esplá, J. Ben-Abda, J. A. Pérez-Álvarez. Date palm by-products as a new ingredient for the meat industry: application to pork liver pâté, Meat Science, Vol. 93, 880-887, 2013.

[21] W. Al-Shahib, R. J. Marshall. Dietary fibre content of dates from 13 varieties of date palm Phoenix dactylifera L., International Journal of Food Science and Technology, Vol. 37, 719-721, 2002.

[22] A. Amorós, M. T. Petrel, M. S. Almansa, M. A. Botella, P. J. Zapata, M. Serrano. Antioxidant and nutritional properties of date fruit from Elche grove as affected by maturation and phenotypic variability of date palm, Food Science and Technology International, Vol. 15, 65-72, 2009.

[23] M. Al-Farsi, C. Alasalvar, A. Morris, M. Baron, F. Shahidi. Compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman, Journal of Agricultural and Food Chemistry, Vol. 53, 7586-7591, 2005.

[24] E. S. Bautista, R. S. Dahiya, M. L. Speck. Identification of compounds causing symbiotic growth of Streptococcus thermophilus and Lactobacillus bulgaricus in milk, Journal of Dairy Research, Vol. 33, 299-307, 1966.

[25] M. Al-Farsi, C. Alasalvar, M. Al-Abid, K. Al-Shoaily, M. Al-Amry, F. Al-Rawahy. Compositional and functional characteristics of dates, syrups, and their by-products, Food Chemistry, Vol. 104, 943-947, 2007.

[26] M. H. Al-Farsi, C. Y. Lee. Nutritional and functional properties of dates: a review, Critical Reviews in Food Science and Nutrition, Vol. 48, No. 10, 877-887, 2008.

[27] A. Mansouri, G. Embarek, E. Kokkalou, P. Kefalas. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera), Food Chemistry, Vol. 89, 411-420, 2005.

[28] C. J. Brady. Fruit ripening, Annual Review of Plant Physiology, Vol. 38, 155-178, 1987.

[29] N. Chaira, A. Mrabet, A. Ferchichi. Evaluation and antioxidant activity, phenolics, sugar and mineral contents in date palm fruits, Journal of Food Biochemistry, Vol. 33, 390-403, 2009.

[30] MM. Al-Farsi, C. Alasalvar, A. Morris, M. Baron, F. Shahidi. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman, Journal of Agricultural and Food Chemistry, Vol. 53, 7592-7599, 2005.

[31] E. A. Amira, S. E. Behija, M. Beligh, L. Lamia, I. Manel, H. Mohamed, A. Lotfi. Effects of the ripening stage on phenolic profile, phytochemical composition and antioxidant activity of date palm fruit, Journal of Agricultural and Food Chemistry, Vol. 60, 10896-10902, 2012.

[32] Z. Benmeddour, E. Mehinagic, D. Le Meurlay, Louaileche. Phenolic composition and antioxidant capacieties of ten Algerian date (Phoenix dactylifera L.) cultivars: a comparative study, Journal of Functional Foods, Vol. 5, 346-354, 2013.

[33] Y. J. Hong, F. A. Tomás-Barberan, A. A. Kader, A. E. Mitchell. The flavonoid glycosides and procyanidin composition of Deglet Noor dates (Phoenix dactylifera), Journal of Agricultural and Food Chemistry, Vol. 54, 2405-2411, 2006.

[34] K. E. Heim, A. R. Tagliaferro, D. J. Bobilya. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships, The Journal of Nutritional Biochemistry, Vol. 13, 572-584, 2002.

[35] N. Cotelle. Role of flavonoids in oxidative stress, Current Topics in Medicinal Chemistry, Vol. 1, 569-590, 2001.

[36] J. Dai, R. J. Mumper. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, Molecules, Vol. 15, 7313-7352, 2010.

[37] D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges, A. Crozier. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases, Vol. 18, No. 14, 1818-1892, 2013.

[38] D. Huang, B. Ou, R. L. Prior. The chemistry behind antioxidant capacity assays, Journal of Agricultural and Food Chemistry, Vol. 53, No. 6, 1841-1586, 2005.

[39] Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López, E. Sendra, E. Sayas-Barberá, J. A. Pérez-Álvarez. Antioxidant properties of pomegranate (Punica granatum L.) bagasses obtained as co-product in the juice extraction, Food Research International, Vol. 44, No. 5, 1217-1223, 2011.

[40] P. K. Vayalil. Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Aracaceae), Journal of Agricultural and Food Chemistry, Vol. 50, 610-617, 2002.

[41] M. Viuda-Martos, J. Fernández-López, E. Sayas-Barberá, E. Sendra, J. A. Pérez-Álvarez. Physicochemical characterization of the orange juice waste water of a citrus by-product, Journal of Food Processing and Preservation, Vol. 35, 264-271, 2011.

[42] M. Chandrasekaran, A. H. Bahkali. Valorization of date palm (Phoenix dactylifera) fruit processing by-products and wastes using bioprocess technology – Review, Saudi Journal of Biological Sciences, Vol. 20, 105-120, 2013.

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PUBLICACIÓN 3

Use of date (*Phoenix dactylifera* L.) blanching water for reconstituting milk powder: Yogurt manufacture

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Use of date (*Phoenix dactylifera* L.) blanching water for reconstituting milk powder: Yogurt manufacture

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A B S T R A C T

The processing of dates yields high volumes of blanching water. The use of such blanching water for reconstituting skim milk powder to produce low fat yogurt was studied. Physicochemical properties and antioxidant activity of two date cultivars (Medjoul and Confitera) blanching water were determined. Quality characteristics of yogurts (control, Medjoul and Confitera) were evaluated during 28 days of refrigerated storage. Results showed that Confitera blanching water is considered a good source of natural antioxidants and organic acids, and has a promising future as a functional ingredient, whereas Medjoul blanching water had a high content of sugars. Regarding yogurt characteristics: Confitera yogurts presented highest populations of lactic acid bacteria, and gave soft gels of weak structure, Medjoul yogurts presented higher firmness and sensory scores than Confitera.

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Keywords: Date; Yogurt; By products; Blanching water; Antioxidant activity

1. Introduction

The food industry has a huge consumption of water, but very limited reuse has been taken place so far, especially in the development of new food applications; main reasons for these limitations are legislations constraints and hygienic concerns (Casani et al., 2005). Nowadays there is a growing interest in the reuse and valorization of water due to environmental problems and economic aspects. Some of the presently accepted direct reuses are initial washing of vegetables, scalding water of meat and poultry, and conveyance of unprocessed products (Rajkowski et al., 1996). Water considered as by-product from vegetable-food processing industry could result from several steps such as cleaning, blanching fruits or vegetables, washing water during the extraction of dietary fiber. The possibility of successfully reusing this water in the food industry, although represents a great challenge, would help in enhancing the economic development and ecoefficiency. The most important aspect is to select an appropriate treatment system to ensure the necessary microbiological and chemical quality. Recently, Viuda-Martos et al. (2009) successfully incorporated citrus waste water derived from the extraction of dietary fiber as an ingredient in a meat product increasing its oxidative stability and therefore extending its shelf-life. Water extracts from other fruits like figs (Yang et al., 2009), citrus fruits (Khuwijitjaru et al., 2008; Xu et al., 2008) and Doum palm fruit (Hsu et al., 2006) have a powerful antioxidant activity.

Date palm fruits (*Phoenix dactylifera* Linnaeus) are eaten fresh, dry, or variously processed. The variety of date products include date honey, date sugar, date vinegar and date wine (made from date fruit juice). Date juice (main product), pressed cake (by-product), and date syrup are the most commonly commercialized date products (Kwaasi, 2003). Dates can play a major role in human nutrition and health because of their wide range of nutritional functional components. They are rich in antioxidant nutrients, including selenium, phenolics, and carotenoids (Al-Farsi and Lee, 2008). Dates are a good source of rapid energy due to their high carbohydrate content (~70–80%), mainly fructose and glucose which are easily absorbed by the human body (Al-Farsi et al., 2007). Several studies have been carried out focusing on the functional properties of dates and its by-products. Al-Shahib and Marshall (2002) determined the dietary fiber content in dried dates from 13 varieties of date palm that ranged from 8.1 to 12.7 (% dry matter). Wu et al. (2004) reported that Deglet Nour and Medjoul

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varieties have a high phenolic content. Recently Amorós et al. (2009) proved the high quality of dates from Elche palm grove because of their low acidity and astringency, and presence of natural antioxidants.

There are an increased number of scientific and clinical evidences supporting the healthy benefits of fermented milk products. Such studies report that probiotics, prebiotics, symbiotic and associated ingredients provide an innovative dimension to cultured dairy products (Khurana and Kanawjia, 2007). Yogurts have been enriched with several physiologically active ingredients that provide specific health benefits beyond basic nutrition, like omega-3 fatty acids (McCowen et al., 2010), phytosterols (Hansel et al., 2007) antioxidants food ingredients such as green and black teas (Jaziri et al., 2009), aqueous vegetable extracts (Cossu et al., 2009; Karaaslan et al., 2011) and fibers (Sendra et al., 2008) with the main purpose to develop new functional dairy products. The reason to incorporate ingredients with antioxidant activity is to enhance the functionality and antioxidant activity of these foodstuffs and in this way to improve consumer's protection against chronic diseases such as cancer and cardiovascular disease (Jiménez et al., 2008).

The aim of this study was to evaluate the direct use of blanching water from two cultivars of date fruits in a set style yogurt on yogurt quality during 28 days of refrigerated storage.

2. Materials and methods

2.1. Materials

Non-commercial Medjoul date and Confitera date fruits were obtained from 'Estación Phoenix' (Elche, Alicante, Spain). Dates ripen in four stages known throughout the world by their Arabic names: "kimri" (unripe; moisture content of 85%), "khalal" (full-size, crunchy; 50–60% moisture), "rutab" (ripe, soft; 35–40% moisture) and "tamr" (ripe, reduced moisture about 20%) (Biglari et al., 2008). Medjoul dates were at the "tamr" stage and Confitera dates were at "rutab" stage, as they are usually harvested at those stages. The pH of blanching water was 6.89 and 6.34 for Medjoul and Confitera date fruits, respectively, and the °Brix values were 7.37 and 2.07, for MBW and CBW. Dates which were going to be processed to date paste were used. Date fruits were washed under running water at 40 °C to remove dust and macroscopic contamination, and afterwards scalded at 100 °C (1:1 water/fruit) for 3 min and immediately divided into date paste (seeds, pulp, peels) and liquid. Blanching water from dates was filtered through cotton gauzes to remove particles and was kept frozen until use. Prior to yogurt manufacture, blanching water was pasteurized in a bath at 80 °C during 30 min to neither affect the quality nor the safety of the yogurt.

For all the study the same batch of skim milk powder was used (34 g 100 g^{-1} protein, 52 g 100 g^{-1} lactose, 1 g 100 g^{-1} fat, 6.8 g 100 g^{-1} ash, 5.2 g 100 g^{-1} moisture; Central Lechera Asturiana, CAPSA, Granada-Siero, Spain). Commercial starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Ezal© MY900, Rhodia Food-Danisco A/S, Sassenage, France) were used at the concentrations recommended by the suppliers. Only for batches prepared for sensory analysis 8% of sucrose was added to each mix to each type of yogurt to sweeten the yogurt.

2.2. Yogurt preparation

The following types of yogurt were prepared: control yogurt, Medjoul yogurt and Confitera yogurt. Skim milk powder was reconstituted with deionized water which is a common industrial practice (Augustin et al., 2003) at 15 g 100 mL^{-1} for control yogurt; for the enriched yogurts, Medjoul blanching water (MBW) and Confitera blanching water (CBW) were replaced as deionized water and reconstituted at the same rate, respectively. Reconstituted skim milk (RSM) was poured into 1-L Pyrex© flasks, closed and immersed into a water bath for heat treatment at 80 °C for 30 min, followed by immersion in ice-water baths to cool down to 43 °C, at this point the starter culture was added and gently shaken. The inoculated mix was poured into 100 mL polystyrene cups of yogurt, capped and incubated at 43 °C to reach pH 4.7, and then cooled down to 4 °C.

2.3. Raw materials analysis

2.3.1. pH and °Brix

The pH was measured using a pH-meter (model pH/Ion 510, Eutech Instruments Pte Ltd., Singapore). The levels of soluble solids of raw material, expressed as °Brix, were measured using a refractometer (Mod. DR-101, Coseta S.A., Barcelona, Spain). Both measurements were taken at 20 °C.

2.3.2. Determination of organic acids and sugars

Contents of organic acids and sugars in blanching water and reconstituted skim milk powder were analyzed. Samples (5 g) were homogenized in 10 mL ultrapure water acidified with 0.1% phosphoric acid H_3PO_4 and shaken vigorously (IKA® T25 digital ULTRA-TURRAX®, IKA® Werke Staufen, Germany) for 20 s at 13,500 rpm and centrifuged for 20 min at 15,000 rpm at 4 °C (Centrifuge Sigma 3-16 PK 10330 rotor 12158-H 25° angle, Shropshire, United Kingdom). The supernatants fluids were filtered through 0.45 μm membrane filters (Millipore Corporation, Bedford, USA). Samples (10 μL) were injected into a cation exchange column (Supelcogel C-610 H, 300 × 7.8 mm, Supelco, Bellefonte) with a precolumn (Supelguard-H, 50 × 4.6 mm, Supelco, Bellefonte), using 0.1% H_3PO_4 as mobile phase, at an operating flow rate of 0.5 mL min^{-1}. A Hewlett Packard HP-1100 instrument (Woldbronn, Germany) coupled with two detectors: DAD G1315A (set at 210 nm) and RID G-1362 A was used. Standards of organic acids, monosaccharides and oligosaccharides were obtained from Supelco. Samples were run at 30 °C and the run time was 30 min (Doughty, 1995). Peaks were identified by comparison with retention time of the standards, and quantified by regression formula obtained with the standards.

2.3.3. Measurement of total phenol content, total flavonoid content and antioxidant activity

Reagents and methods used were according to Viuda-Martos et al. (2010a).

The total phenol content (TPC) was determined using Folin-Ciocalteu's reagent (Singlenton and Rossi, 1965). For total flavonoid content (TFC), the method based on Blasa et al. (2005) was used. Antioxidant activity was measured by three methods: the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined following the method of Viuda-Martos et al. (2010a); the amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. The ferric reducing power (FRAP) was determined by using the potassium ferricyanide-ferric chloride method

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(Oyaizu, 1986); the ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and Rajini (2004); to determine the concentration needed to obtain 50% chelating effect (EC_{50}), the percentage of chelating effect was plotted against sample concentration.

2.4. Yogurt analysis

2.4.1. Physicochemical analysis

2.4.1.1. *Color measurements*. The CIEL*a*b* color space of yogurts was studied, the following color coordinates were determined: lightness (L^*), redness (a^*, +/− red-green), and yellowness (b^*, +/− yellow-blue). Color determinations were made at 12 ± 2 °C by means of a Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer, with a liquid accessory CR-A70 (Minolta Camera Co., Osaka, Japan), with illuminant D_{65} and an observer of 10°.

2.4.1.2. *Texture analysis*. Penetration test was performed with a Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, England) interfaced to a personal computer (Windows-based Software Texture Expert 1.22, Surrey, England) and a 5-kg load cell. Constant speed penetration tests were performed directly on cylindrical containers (4.5 cm diameter × 4 cm height). All instrumental texture analyses were conducted on chilled (7 °C) samples. A cylindrical probe 10 mm diameter ebonite (P-10) was introduced 15 mm into the samples at a speed of 1 mm s^{-1}. From the force-versus-time curves, values for the maximum force (N) were calculated as force at a distance of 15 mm (F_{max}). Triplicate measures for each yogurt were performed.

2.4.2. Rheological measurements

The rheological parameters were measured by oscillatory testing, in triplicate at 7 °C, by means of a rheometer model Rheostress 600 (Software Rheowin-RS600 version 3.2, Haake, Karlsruhe, Germany) according to Sendra et al. (2010). Tests were performed on slices of 1.2 ± 0.2 mm thickness and 35 mm diameter obtained using a sharp blade. It was verified that all samples were within the range of linear viscosity. The geometry used was plate and plate with serrated platens (35 mm diameter, 1 mm gap). The measurements were conducted in triplicate. A frequency test was run: frequency sweep from 0.01 to 10 Hz at a constant stress of 0.4 Pa with three cycles of measurements in both tests. The data obtained through this type of dynamic measurements are the contributions to the internal structure of the sample from the elastic and viscous portions of flow: G' and G'' (Pa), respectively, the complex viscosity η^* (Pa s), the tan(δ) which is equal to G''/G' and the deformation (γ).

2.4.3. Sensory evaluation

A sample group of 30 consumer panelists was recruited at the Miguel Hernandez University, Alicante, Spain. All sensory work was carried out in the sensory laboratory at the University, which fulfills requirements according to the International Standards (ISO, 1988). A panel of three experts defined the attributes to be evaluated in yogurts. Panelists were informed and agreed to taste the samples before the tests, and they were informed of the type of product being tested and asked about yogurt consuming habits. Tap water was provided between samples to cleanse the palate. White plastic cups with 40 mL of yogurt at 10 °C were provided. The consumer study was carried out during three different sessions. In each session, consumers tested all three yogurts; the sample presentation order was randomized for each panelist. A seven-point hedonic scale (from dislike extremely to like extremely) was used to rate the following parameters: appearance, creaminess, sweetness, astringency, sourness, flavor and overall acceptance.

2.4.4. Microbiological analysis

Microbial analyses of yogurts were carried out at days 1, 7, 14, 21 and 28 at 4 °C of cold storage. Streptococci were estimated by counting on M-17 agar (Scharlau, Barcelona, Spain) incubated aerobically at 37 °C for 24 h. Lactobacilli were estimated by culture in MRS agar (Cultimed, Panreac, Castellar del Vallés, Barcelona, Spain) under anaerobic incubation at 37 °C for 48 h. Enumerating of yeasts and molds was done on Rose Bengal agar with Chloramphenicol (Scharlau, Barcelona, Spain) using the spread plate technique and plates were incubated under aerobic conditions at 28 °C for 5 days.

2.4.5. Organic acids and sugars analysis

Organic acids and sugars from yogurts were analyzed during cold storage, as previously described in Section 2.3.2.

2.5. Statistical analysis

PASW Statistics 18 software package, SPSS Inc., IBM (Chicago, IL, USA) was used. General Linear Model procedure was tested to evaluate factors (water source and storage time effect on yogurt samples). Tukey's pair wise comparisons test was used for means comparison (95% confidence level). For sensory data, texture and color of yogurts and blanching water samples one-way ANOVA test was used as type of yogurt was the only factor. The whole experiment was independently run three times.

3. Results and discussion

3.1. Raw material characterization

3.1.1. Organic acid and sugar profile in raw materials

Table 1 shows the profile of organic acids and sugars of date blanching water and reconstituted skim milk powder. Regarding blanching water, CBW had the highest total acidity content ($P < 0.05$) and MBW yielded the highest total soluble solids content which was significantly different to that of CBW. As a consequence there is an increase in total soluble solids in the yogurt mix due to MBW and CBW use.

Amorós et al. (2009) analyzed dates from Elche palm grove in different maturation stages for organic acid content. Malic acid was the major organic acid in date fruit and increased during ripening, with a final concentration ranging from 0.13 to 0.21 g 100 g^{-1} depending on date palm. Citric, succinic, and fumaric acids were also reported to be present at lower concentrations. In our study, succinic acid is the predominant acid in blanching water. Ascorbic acid was not present in blanching water although a positive concentration was found in the date paste (12.65 and 11.14 mg 100 g^{-1} for Medjoul and Confitera pastes, respectively) so it might be possible that blanching could cause thermal inactivation. Al-Farsi et al. (2005) reported similar contents of succinic acid for three sun-dried date varieties ranging from 196 mg 100 g^{-1} to 702 mg 100 g^{-1} but presented a substantial difference in malic acid content. They identified six organic acids among which malic acid was the predominant (1265–1396 mg 100 g^{-1}) contributing 52.2–65.2% to the total organic acid content. They reported lower amounts

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Table 1 – Organic acids (mg 100 g^{-1}) and sugars (g 100 g^{-1}) profile of reconstituted skimmed milk and dates blanching water.

Organic acids (mg 100 g^{-1})	RSM	Dates	
		MBW	CBW
Oxalic acid	3.60 (0.44)*	0.18^{a} (0.02)	3.30^{b} (0.15)
Citric acid	233.41 (24.3)	8.10^{a} (0.90)	54.05^{b} (1.96)
Malic acid	2.47 (0.69)	13.15^{b} (0.86)	17.09^{b} (0.10)
Ascorbic acid	0.37 (0.11)	N.d.	N.d.
Succinic acid	N.d.	118.57 (24.51)	212.30 (21.20)
Lactic acid	122.23 (3.93)	N.d.	N.d.
Formic acid	N.d.	4.76^{a} (0.20)	40.31^{b} (2.31)
Acetic acid	14.85 (1.87)	2.79^{a} (6.04)	15.29^{b} (2.98)
Fumaric acid	3.36 (0.12)	7.65^{a} (1.54)	40.34^{b} (4.87)
Total acidity (g 100 g^{-1})	0.38 (0.07)	0.16^{a} (0.03)	0.38^{b} (0.03)
Sugars (g 100 g^{-1})			
Sucrose	N.d.	0.71 (0.22)	0.86 (0.08)
Glucose	N.d.	2.90^{b} (0.62)	0.18^{a} (0.01)
Fructose	N.d.	2.53^{b} (0.56)	0.17^{a} (0.00)
Lactose	6.40 (0.65)	N.d.	N.d.
Total sugar (g 100 g^{-1})	6.40 (0.65)	6.14^{b} (1.39)	1.20^{a} (0.08)

RSM: reconstituted skimmed milk; MBW: Medjoul blanching water; CBW: Confitera blanching water; N.d.: not detected. Values with different superscript letters within the same line significantly differ (Tukey's test, $P < 0.05$).

* Values expressed are means of three parallel measurements where parentheses denote standard error ($n = 9$).

of isobutyric acid, citric acid, oxalic acid, and formic acid. Organic acids are intermediates of metabolic process in dates and are directly involved in growth, maturation and senescence, so the presence of some acids in higher amount could be attributed to their state of ripening.

Al-Farsi and Lee (2008) reviewed the nutritional constituents from over 80 date fruit references concluding that fructose, glucose and sucrose were the only sugars detected in fresh and dried dates reporting average contents in fresh dates of 19.4, 22.8 and 4.03 g 100 g^{-1}, respectively, with contents of all sugars increased in dried dates. The profile and relative content of sugars changed according to date cultivar. As can be seen in Table 1 MBW contained 15 times more glucose and fructose than CBW. In MBW glucose and fructose contents were 4 times high than sucrose. The content of sucrose in CBW was almost 5 times the glucose and fructose content. This variability in the sucrose content is due to the different stage of ripening of date fruits.

In skim milk unique sugar detected was lactose and main organic acid identified was citric acid (we assume that citrate is included in the detection) followed by lactic and acetic acids and small amounts of other organic acids (oxalic, fumaric, malic and ascorbic acids). A huge peak was detected at the retention time of tartaric acid, but it has not been included in the table as we have reasonable doubts about the truly identification. If quantified as tartaric, the estimated concentration would be of about 400 mg 100 g^{-1}. Our results were higher than those reported by Mullin and Emmons (1997) which found mean values of 156.5 mg 100 g^{-1} and 3.98 g 100 g^{-1} for citric acid and lactose in milk.

3.1.2. *Total phenol content, total flavonoid content and antioxidant activity*

The total phenol (TPC) and flavonoid (TFC) contents of Medjoul and Confitera blanching water are presented in Table 2; their presence in the blanching water could be explained by leaching during the blanching operation. Results showed that CBW had the highest TPC and TFC content ($P < 0.05$), 492 mg GAE L^{-1} and 68.75 mg RE 100 g^{-1}, respectively, which corresponds with the early maturation stage of Confitera date fruit since Myhara et al. (2000) reported that phenolic substances were high in the inedible "kimri" stage of dates and declined progressively as dates matured to "tamr" stage. No data is available in the literature regarding TPC of date blanching water but our results (Table 2) found in the blanching water were similar than those in date fruits reported by Mansouri et al. (2005) and Chaira et al. (2009) who showed that the phenol content can vary from 2.49 to 8.36 mg GAE 100 g^{-1} in some Algerian date varieties (methanolic extracts) and from 3.879 to 9.706 mg GAE 100 g^{-1} in 10 Tunisian date varieties (aqueous and methanolic extracts). However, these concentrations were lower than those observed by Wu et al. (2004) who showed that the phenol content varied from 572 to 661 mg GAE 100 g^{-1} in two dates from U.S.A. (the sum of lipophilic and hydrophilic content), Saafi et al. (2009) who analyzed four Tunisian date varieties and obtained values ranging from 209.42 mg GAE 100 g^{-1} to 443.73 mg GAE 100 g^{-1} (methanolic extracts) and Al-Farsi et al. (2007) who reported values between 172 and 246 mg GAE 100 g^{-1} in three Oman date varieties (aqueous methanolic extracts). In addition to the type of extraction, various factors such as variety, growing condition, maturity, season, geographic origin, storage conditions, among others, might be responsible for the observed differences (Al-Farsi et al., 2007). Gad et al. (2010) have developed a yogurt fortified with date extract which have demonstrated to possess highest antioxidant activities compared with those of control yogurt, probably attributed to the date enrichment.

The radical scavenging capacity of the raw materials was tested using the 'stable' free radical, DPPH (Table 2). CBW showed the strongest ($P < 0.05$) radical scavenging effect at 7.5 g 100 g^{-1} over all samples tested, which is in accordance with the higher phenol content. In the DPPH assay, the increased antioxidant activity is reflected in a lower IC_{50}. Viuda-Martos et al. (2010a) reported IC_{50} values of 0.042% and 0.053% for ascorbic acid and butylated hydroxytoluene (BHT) as positive controls. Vayalil (2002) reported the potent

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Table 2 – Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of Medjoul and Confitera blanching water at different concentrations (A = 2.5%, B = 5%, C = 7.5%, D = 10%).

Type of raw material	TPC (mg GAE L^{-1})	TFC (mg RE 100 g^{-1})	DPPH inhibition (%)					FRAP TEAC (mM Trolox L^{-1})				FIC chelating effect (%)				
			A	B	C	D	IC_{50}	A	B	C	D	A	B	C	D	EC_{50}
MBW	167.14 (2.38)*	22.30 (0.63)	4.87 (0.41)	5.83 (0.46)	5.79 (0.14)	6.76 (0.19)	41.51	0.08 (0.02)	0.08 (0.00)	0.12 (0.00)	0.19 (0.04)	1.45 (0.03)	3.64 (0.02)	4.45 (0.04)	9.73 (0.04)	17.07
CBW	492.54 (7.14)	68.75 (2.08)	9.60 (0.28)	15.16 (0.51)	17.37 (0.14)	17.18 (0.23)	61.75	0.24 (0.01)	0.41 (0.03)	0.57 (0.02)	0.81 (0.02)	17.82 (0.10)	26.73 (0.04)	32.82 (0.04)	47.18 (0.05)	10.52

MBW, Medjoul blanching water; CBW, Confitera blanching water; GAE, Gallic acid equivalent; RE, Rutin equivalent, TEAC, Trolox equivalent antioxidant capacity.

* Values expressed are means of three parallel measurements where parentheses denote standard error ($n = 9$).

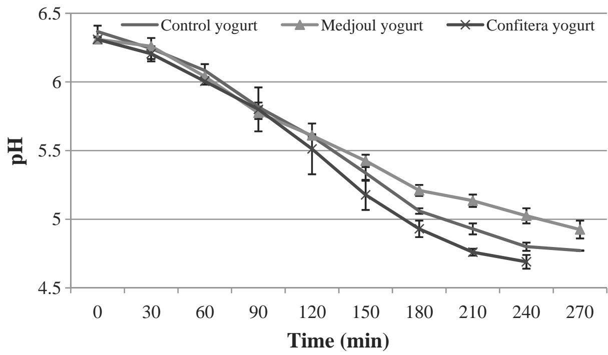

Fig. 1 – Acidification curves of yogurts with dates blanching water. Data are the average ($n = 9$) ± standard error.

antioxidant activity of date fruit by an aqueous extract which inhibited superoxide and hydroxyl radicals. Table 3 also shows the ferric reducing capacity obtained using the FRAP assay. In this study CBW showed the highest values ($P < 0.05$) of inhibition and Trolox equivalent antioxidant capacity (TEAC) of DPPH and FRAP.

MBW and CBW were capable of chelating iron(II) in a concentration-dependent manner (Table 2). CBW presented the highest values of metal chelation ($P < 0.05$) at all concentrations tested, showing the highest value (47.18 ± 0.05%) at 10 g 100 g^{-1} and reflected in a lower EC_{50}. Also results were consistent with DPPH and FRAP assays in which CBW showed higher antioxidant activities than MBW. According to Viuda-Martos et al. (2010a) chelating ability is of great potential interest in the food industry because the transition of metal ions contributes to lipid oxidation, which is the main source of degradation of food products.

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3.2. *Characterization of yogurt with dates blanching water*

3.2.1. *Acidification process*

The enrichment of yogurts with CBW stimulates the fermentation process whereas MBW increased the pH of the final product as compared with control yogurts ($P < 0.05$) (Fig. 1). This could be due to the fact that CBW provides formic acid (40.31 mg 100 g^{-1}) that may stimulate the growth of lactobacilli (Bautista et al., 1966) (Table 1), reducing the time required to obtain the final pH ($P < 0.05$) in 30 min which is confirmed by the high lactic acid content of this type of yogurt the 1st day of cold storage (see Section 3.2.6).

3.2.2. *Physicochemical analysis of yogurts*

3.2.2.1. *Color.* Color is the first sensory characteristic perceived by consumer and tends to modify other perceptions as flavor and aroma (García-Pérez et al., 2005). The addition of MBW and CBW had no effect ($P > 0.05$) on L^* values and b^* coordinate, only a^* was affected by the addition of CBW ($P < 0.05$) (Table 3). All samples had higher values of a^* (less greenness) and b^* (more yellowness) coordinates as compared with control samples, this would be due to the fact that all blanching water (raw material) had brownish color but light enough not to induce major changes in yogurt color. The addition of date paste fiber to yogurt had an obvious effect on color parameters in the study carried out by Hashim et al. (2009).

3.2.2.2. *Texture.* CBW yogurts had significantly ($P < 0.05$) lower F_{max} values (23.90 ± 1.16 N, respectively) than control and

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Table 3 – Color and texture of yogurts with dates blanching water.

Type of yogurt	L^*		a^*		b^*		F_{max} (N)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	84.27	1.11	-2.74^{a}	0.06	5.34	0.16	32.56^{b}	1.04
Medjoul	85.46	1.91	$-2.42^{a,b}$	0.07	5.56	0.17	32.60^{b}	0.57
Confitera	82.71	0.78	-1.59^{b}	0.18	6.66	0.24	23.90^{a}	1.16

Results expressed are means ± standard error of three parallel measurements ($n = 9$). Values with different superscript letters within the same column significantly differ (Tukey's test, $P < 0.05$). F_{max} (N): firmness.

MBW yogurts (32.56 ± 1.04 N and 32.60 ± 0.57 N), which means that the structure of control and MBW is harder than that of CBW yogurts. The increased total solids content of MBW yogurts (6.14%) may affect the firmness of the product. The sudden pH decreased caused by CBW addition to skimmed milk (6.6, whereas control and MBW was about 6.89), followed by the heat treatment may have caused a pre-acidification effect. In such conditions, Peng et al. (2009) reported increased solubilization of colloidal calcium phosphate, increased early loss of colloidal calcium phosphate cross links and the production of weak gels, as observed in our study for CBW yogurts.

3.2.3. *Rheology of set style yogurts enriched with dates blanching water*

The studied yogurts remained in the linear viscoelastic region over the whole range of frequencies tested (0.01–1 Hz). G', G'', η^*, tan(δ) and γ at 0.1 Hz were selected to run statistical analysis of the data. Although there were significant differences between yogurts through the storage period such differences may be considered as irrelevant, so presented results are the average of all measurements taken for each type of yogurts. Mean values for rheological parameters for control, Medjoul and Confitera yogurts were as follows: G' (1065 ± 162 Pa, 823 ± 25 Pa and 759 ± 72 Pa), G'' (223 ± 37 Pa, 185 ± 6 Pa and 172 ± 16 Pa), η^* (1735 ± 264 Pa s, 1342 ± 39 Pa s and 1239 ± 118 Pa s); tan(δ) (0.219 ± 0.004, 0.225 ± 0.003 and 0.226 ± 0.005), and γ (0.00039 ± 0.00011, 0.00052 ± 0.00001 and 0.00032 ± 0.00004).

The elastic modulus dominated ($G' > G''$) the at rest response of the samples, indicating an elastic characteristic that gives better stability during storage and implying that at the frequencies tested, the yogurts behaved as a solid. In general, moduli (G' and G'') increased with increased frequency showing concentrate solution characteristics (Staffolo et al., 2004). A low value of the phase angle (tan δ) indicates that the gel has a predominant elastic character (more solid like) (Singh and Muthukumarappan, 2008). Our experimental values for tan(δ) ranged from 0.210 to 0.243 which pointed to a concentrated amorphous polymer (Steffe, 1996).

Storage time for 15 days did not cause significant changes in the viscoelastic properties of yogurts. However, on 21st and the 28th day, tan(δ) was found to be lower ($P < 0.05$) from that on 1st and 7th day pointing to a hardening of the structure during cold storage, probably related to the increase in lactic acid (see Section 3.2.6). Regarding yogurt type, control yogurts presented higher moduli values than enriched yogurts and also the highest viscosity, followed by Medjoul yogurts. Controls were more solid like (lower tan(δ)).

From both texture (Table 3) and oscillatory testing it can be concluded that few differences were detected among control and Medjoul enriched yogurts (the ones with the highest soluble solids content), whereas the use of Confitera blanching water softened the gels and weakened the structure. The faster acidification rate caused by CBW (Fig. 1) use may have caused large clusters of micelles that did not disperse during gelation occurred by fermentation, so CBW yogurts are expected to have large clusters of casein and large pores, a coarse network that yields weak gels (Peng et al., 2009).

3.2.4. *Sensory analysis*

Consumers' affective test, such as the test run in this study, provide useful information on the future commercial potential of a new developed food (Cardarelli et al., 2008). Fig. 2 shows the results obtained for the sensory evaluation of the yogurts. There were significant differences in appearance, flavor and overall acceptability between control and enriched yogurts. Regarding appearance, the panelists judged negatively the addition of blanching water which is supported by the instrumental measured a^* and b^* coordinates. In the case of creaminess, differences detected in texture by instrumental means were not detected by sensory evaluation. Results of sweetness and sourness obtained by sensory evaluation are quite different from instrumental measurements; panelists scored those attributes higher in control yogurts while by instrumental means Confitera yogurts had the highest acidity and Medjoul yogurts had the highest sugar content. The enrichment of yogurt with MBW improved flavor perception ($P < 0.05$). Control and MBW yogurts showed the highest values for overall acceptability (4.93 ± 0.20 and 4.45 ± 0.32 points). Hashim et al. (2009) reported that yogurt fortified with up to 3% date fiber had similar acidity, sweetness, firmness, smoothness and overall acceptance ratings as the control yogurts. Also, Viuda-Martos et al. (2009, 2010b) had successfully incorporated blanching water from citrus industry to a meat product with good sensory scores. Control followed by MBW yogurts obtained best acceptability scores.

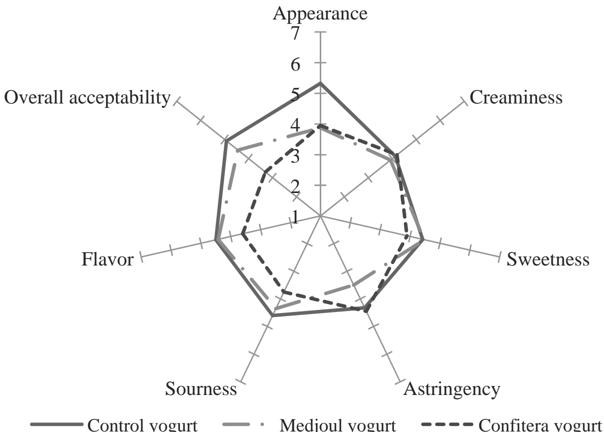

Fig. 2 – Sensory evaluation of yogurts with dates blanching water (7-point hedonic scale). Data are the average ($n = 36$).

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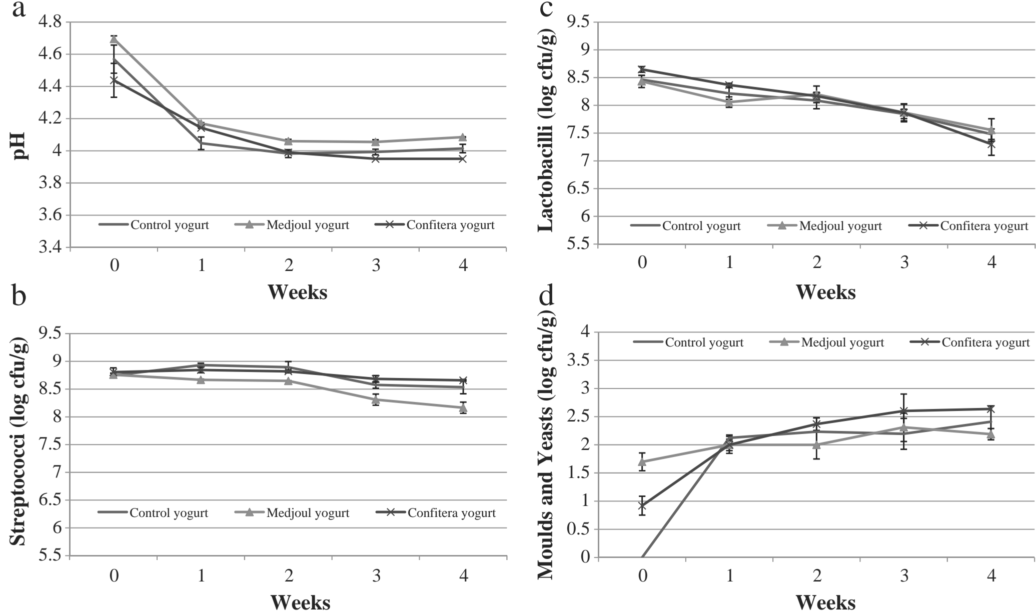

Fig. 3 – Evolution of (a) pH, (b) counts of streptococci, (c) counts of lactobacilli, and (d) counts of moulds and yeasts of yogurts stored at 4 °C for 28 days. Data are the average ($n = 9$) ± standard error.

3.2.5. *Evolution of pH and microbial counts during cold storage*

Higher pH values were observed ($P < 0.05$) in MBW yogurts than in control and CBW yogurts. During storage at 4 °C significant variations in pH values were observed (Fig. 3a) most probably due to continued fermentation by the lactic acid bacteria (Dave and Shah, 1997).

Ranging between 7.15 and 8.85 log CFU g^{-1}, all the counts of streptococci and lactobacilli largely exceed the minimum requirements of viable cells at the time of consumption by Spanish legislation (10^7) (R.D. 179/2003, B.O.E. 18/02/03) throughout the entire storage period. Levels of *S. thermophilus* were higher than *L. bulgaricus*. Viability of *S. thermophilus* and *L. bulgaricus* in yogurt is pH dependent (Nogueira et al., 1998). Usually, numbers of *S. thermophilus* increase in yogurts with an initial pH greater than 4 until the pH decrease below 4, and the numbers then diminish rapidly. Numbers of *L. bulgaricus* either remain constant or increase for the first 10–20 days in yogurts with an initial pH greater than 4 and then decrease. In the present study counts of *S. thermophilus* were significantly higher in control and CBW yogurts than in MBW yogurts (Fig. 3b) and counts of *L. bulgaricus* were highest ($P < 0.05$) in CBW yogurts (Fig. 3c) which is correlated by the low pH of the product and was explained by the natural presence of formic acid in the original CBW. Finally, numbers of *L. bulgaricus* decreased faster than did those of *S. thermophilus* which remained stable throughout the storage period.

Since milk is pasteurized before yogurt production, the presence of yeasts in yogurt is caused by recontamination processes during manufacture, and can be a problem in fruit-containing yogurts (Nogueira et al., 1998). Numbers of yeasts and molds were significantly higher in CBW yogurts than in control yogurts. Counts of yeasts and molds increased ($P < 0.05$) in yogurts during cold storage (Fig. 3d) and the highest level was 4.33×10^2 CFU g^{-1} at day 28 in CBW yogurts. Confitera dates have ripening problems and a high percentage of the fruits are not suitable for direct consumption due to cracks and molds growth, which may be in the origin of the higher molds and yeast population in such yogurts.

3.2.6. *Evolution of organic acids and sugar content in yogurts during cold storage*

Quantitative determination of organic acids is important for monitoring bacterial growth and activity, but also because they are significant as natural preservatives and for sensory characteristics of the product (Serra et al., 2009). All organic acids detected were significantly ($P < 0.05$) affected by the type of yogurt. MBW yogurts had higher concentration of oxalic acid ($P < 0.05$) (values increased during storage time from 2.59 to 6.22 mg 100 g^{-1}) than CBW yogurts and control yogurts (values decreased from 2.12 to 1.87 mg 100 g^{-1} for CBW yogurts and from 1.49 to 1.14 mg 100 g^{-1} for control yogurts throughout the storage period); however differences were very small. Citric acid was also present with significant differences among enriched and control yogurts ($P < 0.05$) being lower in control. During cold storage, in control yogurts, citric acid content varied from 198.94 to 187.65 mg 100 g^{-1}; for the enriched ones the content ranged from 228.27 to 248.21 mg 100 g^{-1} and 256.54 to 221.45 mg 100 g^{-1} for MBW and CBW yogurts, respectively. Citric acid is usually present in milk as a product of bovine metabolism; also citric acid is known to be utilized during the fermentation process, but insignificant utilization of this acid was observed during storage as reported by (Serra et al., 2009). Lactic acid was the major organic acid. CBW yogurts had higher values of lactic acid (1.54 ± 0.04 g 100 g^{-1} as mean value) ($P < 0.05$) than control yogurts (1.30 ± 0.03 g 100 g^{-1} as mean value) and this is correlated with the highest counts of lactobacilli in CBW yogurts (Fig. 3b). The content of acetic acid in control yogurts ranged from 11.13 to 16.01 mg 100 g^{-1}, from 20.95 to 16.05 mg 100 g^{-1} for MBW yogurts and from 10.66 to 14.27 mg 100 g^{-1} for CBW yogurts. No significant differences in the content of organic acids were detected in yogurts during storage time except for lactic acid which increased ($P < 0.05$) its concentration on 7th and 28th day with respect to 1st day of cold storage. Again, a huge unidentified peak was observed at the retention time of tartaric acid in all yogurt samples, if calculated based on tartaric standard, its concentration would range from 235 to 320 mg 100 g^{-1} yogurt. Finally total acidity increased in all yogurts during the storage period reaching a

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final acidity (%) of 1.40, 1.84 and 1.75 for control, MBW and CBW yogurts, respectively.

The amount of lactose decreased during storage time. Galactose increased or remained constant in all yogurts ($P > 0.05$) (values ranged from 1.12 to 1.29 mg 100 g^{-1} from control yogurts, from 1.13 to 1.47 mg 100g 100 g^{-1} for MBW yogurts and from 1.26 to 1.30 mg 100 g^{-1} for CBW yogurts). This is because of the cultures used in yogurt fermentation (*L. bulgaricus* and *S. thermophilus*) utilize the glucose moiety of lactose, but not the galactose moiety. Thus, while lactose and glucose content in fermented product decrease, the galactose content remains unchanged (O'Brien, 1999). Lactose content is much lower ($P < 0.05$) in control yogurts than in enriched yogurts (mean values of 3.92 ± 0.14 g 100g 100 g^{-1}, 5.47 ± 0.05 g 100 g^{-1} and 5.16 ± 0.24 g 100 g^{-1} for control, MBW and CBW yogurts, respectively). As dates blanching water provide glucose (sucrose and fructose also) to skim milk, glucose was detected in MBW (156.65 ± 5.19 mg 100 g^{-1} as mean value) and CBW yogurts (41.74 ± 1.15 mg 100 g^{-1} as mean value), but in lower amounts than in the initial products (blanching water). The content of maltohexaose ranged from 84.66 to 73.07 mg 100 g^{-1}, from 143.02 to 116.12 mg 100 g^{-1} and from 90.76 to 88.09 mg 100 g^{-1} for control, MBW and CBW yogurts, respectively. Summarizing, MBW yogurts contained higher amounts of maltohexaose, glucose and galactose than CBW yogurts. These results are consistent with the initial total soluble solids content of the raw material.

4. Conclusions

Blanching water from dates processing have an important content in phenols and flavonoids, that confer interesting antioxidant properties, as well as organic acids and sugars extracted during blanching. The information obtained from the sensory evaluation reveals that Medjoul blanching water is suitable for direct use in the manufacture of yogurts, confirmed by textural and color values. Confitera provides the best antioxidative properties and also enhances lactic acid bacteria growth. Throughout 28 days of cold storage pH decreases, the gel structure is reinforced, lactobacilli counts decrease and yeast and mold populations increase.

In the future it would be interesting to test other food uses for the blanching water or their bioactive components for its antioxidative properties.

References

Al-Farsi, M.H., Lee, C.Y., 2008. Nutritional and functional properties of dates: a review. Crit. Rev. Food Sci. 48, 877–887.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., Shahidi, F., 2005. Compositional and sensory characteristics of three native sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. J. Agric. Food Chem. 53, 7586–7591.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily-K., Al-Amry, M., Al-Rawahy, F., 2007. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem. 104, 943–947.

Al-Shahib, W., Marshall, R.J., 2002. Dietary fibre content of dates from 13 varieties of date palm *Phoenix dactylifera* L. Int. J. Food Sci. Technol. 37, 719–721.

Amorós, A., Petrel, M.T., Almansa, M.S., Botella, M.A., Zapata, P.J., Serrano, M., 2009. Antioxidant and nutritional properties of date fruit from Elche grove as affected by maturation and phenotypic variability of date palm. Food Sci. Technol. Int. 15, 65–72.

Augustin, M.A., Clarke, P.T., Craven, H.M., 2003. Powdered milk: characteristics of milk powders. In: Caballero, B., Finglas, P.M., Trugo, L. (Eds.), Encyclopaedia of Food Science and Nutrition. , 2nd ed. Elsevier Science, London, pp. 4703–4711.

Bautista, E.S., Dahiya, R.S., Speck, M.L., 1966. Identification of compounds causing symbiotic growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk. J. Dairy Res. 33, 299–307.

Biglari, F., Alkarkhi, A.F.M., Easa, A.M., 2008. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem. 107, 1636–1641.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, P.M., Albertini, M.C., Piatti, E., 2005. Raw Millefiori honey is packed full of antioxidants. Food Chem. 97, 217–222.

Cardarelli, H.R., Buriti, F.C.A., Castro, I.A., Saad, S.M.I., 2008. Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially synbiotic petit-suisse cheese. LWT-Food Sci. Technol. 41, 1037–1046.

Casani, S., Rouhany, M., Knøchel, S., 2005. A discussion paper on challenges and limitations to water reuse and hygiene in the food industry. Water Res. 39, 1134–1146.

Chaira, N., Mrabet, A., Ferchichi, A., 2009. Evaluation and antioxidant activity, phenolics, sugar and mineral contents in date palm fruits. J. Food Biochem. 33, 390–403.

Cossu, M., Juliano, C., Pisu, R., Alamanni, M.C., 2009. Effects of enrichment with polyphenolic extracts from Sardinian plants on physico-chemical, antioxidant and microbiological properties of yogurt. Ital. J. Food Sci. 21, 447–459.

Dave, R.I., Shah, N.P., 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Int. Dairy J. 7, 31–41.

Doughty, E., 1995. Separation of acids, carbohydrates and fermentation products by HPLC. Lebensm. Biotechnol. 12, 100–101.

Gad, A.S., Kholif, A.M., Sayed, A.F., 2010. Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. Am. J. Food Technol. 5, 250–259.

García-Pérez, F.J., Lario, Y., Fernández-López, J., Sayas, E., Pérez-Álvarez, J.A., Sendra, E., 2005. Effect of orange fiber addition on yogurt color during fermentation and cold storage. Color Res. Appl. 30, 457–463.

Hansel, B., Nicolle, C., Lalanne, F., Tondu, F., Lassel, T., Donazzolo, Y., Ferrières, J., Krempf, M., Schlienger, J.-L., Verges, B., Chapman, M.J., Bruckert, E., 2007. Effect of low-fat, fermented milk enriched with plant sterols on serum lipid profile and oxidative stress in moderate hypercholesterolemia. Am. J. Clin. Nutr. 86, 790–796.

Hashim, I.B., Khalil, A.H., Afifi, H.S., 2009. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. J. Dairy Sci. 92, 5403–5407.

Hsu, B., Coupar, I.M., Ng, K., 2006. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chem. 98, 317–328.

ISO, 1988. International Standard 8589. Sensory Analysis General Guidance for the Design of Test Rooms. Ref. No. ISO 8589:1988 (E). International Organization for Standardization, Geneva.

Jaziri, I., Ben Slama, M., Mhadhbi, H., Urdaci, M.C., Hamdi, M., 2009. Effect of green and black teas (*Camellia sinensis* L) on the characteristic microflora of yogurt during fermentation and refrigerated storage. Food Chem. 112, 614–620.

Jiménez, A.M., Murcia, M.A., Parras, P., Martínez-Tomé, M., 2008. On the importance of adequately choosing the ingredients of yoghurt and enriched milk for their antioxidant activity. Int. J. Food Sci. Technol. 43, 1464–1473.

Karaaslan, M., Ozden, M., Vardin, H., Turkoglu, H., 2011. Phenolic fortification of yogurt using grape and callus extracts. LWT-Food Sci. Technol. 44, 1065–1072.

Khurana, H.K., Kanawjia, S.K., 2007. Recent trends in development of fermented milks. Curr. Nutr. Food Sci. 3, 91–108.

Khuwijitjaru, P., Chalooddong, K., Adachi, S., 2008. Phenolic content and radical scavenging capacity of kaffir lime fruit

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peel extracts obtained by pressurized hot water extraction. Food Sci. Technol. Res. 14, 1–4.
Kwaasi, A.A.A., 2003. Date palms. In: Caballero, B., Finglas, P.M., Trugo, L. (Eds.), Encyclopaedia of Food Science and Nutrition. , 2nd ed. Elsevier Science, London, pp. 1730–1740.
Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P., 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chem. 89, 411–420.
McCowen, K.C., Ling, P.R., Decker, E., Djordjevic, D., Roberts, R.F., Coupland, J.N., Bistrian, B.R., 2010. A simple method of supplementation of omega-3 polyunsaturated fatty acids: use of fortified yogurt in healthy volunteers. Nutr. Clin. Pract. 25, 641–645.
Mullin, W.J., Emmons, D.B., 1997. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography. Food Res. Int. 30, 147–151.
Myhara, R.M., Al-Alawi, A., Karkalas, J., Taylor, M.S., 2000. Sensory and textural changes in maturing Omani dates. J. Sci. Food Agric. 80, 2181–2185.
Nogueira, C., Albano, H., Gibbs, P., Teixeira, P., 1998. Microbiological quality of Portuguese yogurts. J. Ind. Microbiol. Biotechnol. 21, 19–21.
O'Brien, J., 1999. Sugar profiles of cultured dairy products in the UK. J. Hum. Nutr. Diet. 12, 245–250.
Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315.
Peng, Y., Horne, D.S., Lucey, J.A., 2009. Impact of preacidification of milk and fermentation time on the properties of yogurt. J. Dairy Sci. 92, 2977–2990.
Rajkowski, K.T., Rice, E.W., Huynh, B., Patsy, J., 1996. Growth of *Salmonella* spp. and *Vibrio cholerae* in reconditioned wastewater. J. Food Prot. 59, 577–581.
Saafi, E.B., El Arem, A., Issaoui, M., Hammami, M., Achour, L., 2009. Phenolic content and antioxidant activity of four date palm (*Phoenix dactylifera* L.) fruit varieties grown in Tunisia. Int. J. Food Sci. Technol. 44, 2314–2319.
Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E., Pérez-Álvarez, J.A., 2008. Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiol. 25, 13–21.
Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C., Pérez-Álvarez, J.A., 2010. Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. LWT-Food Sci. Technol. 30, 1–7.
Serra, M., Trujillo, A.J., Guamis, B., Ferragut, V., 2009. Flavour profiles and survival starter cultures of yoghurt produced from high-pressure homogenized milk. Int. Dairy J. 19, 100–106.
Singh, G., Muthukumarappan, K., 2008. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT-Food Sci. Technol. 41, 1145–1152.
Singh, N., Rajini, P.S., 2004. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 85, 611–616.
Singlenton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144–158.
Staffolo, M.D., Bertola, N., Martino, M., Bevilacqua, A., 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. Int. Dairy J. 14, 263–268.
Steffe, J.F., 1996. Rheological Methods in Food Process Engineering. East Lansing, Michigan.
Vayalil, P.K., 2002. Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Aracaceae). J. Agric. Food Chem. 50, 610–617.
Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J.A., 2009. Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. Innov. Food Sci. Emerg. 10, 655–660.
Viuda-Martos, M., Ruiz Navajas, Y., Sánchez-Zapata, Fernández-López, J., Pérez-Álvarez, J.A., 2010a. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Frag. J. 25, 13–19.
Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J.A., 2010b. Effect of adding citrus fibre washing water and rosemary essential oil on the quality characteristics of a bologna sausage. LWT-Food Sci. Technol. 43, 958–963.
Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L., 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric. Food Chem. 52, 4026–4037.
Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., Jiang, P., Ye, X.Q., 2008. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73, C11–C18.
Yang, X.-M., Yu, W., Ou, Z.-P., Ma, H.-L., Liu, W.-M., Ji, X.-L., 2009. Antioxidant and immunity activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. Plant Food Hum. Nutr. 64, 167–173.

PUBLICACIÓN 4

Low fat set yoghurt rich in pomegranate juice: A new antioxidant dairy product

Lorena Trigueros, Manuel Viuda-Martos, Jose Ángel Pérez-Álvarez, Esther Sendra

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PUBLICACIÓN 5

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Article

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Antioxidant activity and interactions protein-polyphenol in a pomegranate (*Punica granatum* L.) yogurt

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1 **ABSTRACT**

2 Pomegranate juice (PGJ) is rich in phenolics which are potent antioxidants but also
3 prone to interact with proteins. A yogurt rich in PGJ (40%) made from arils was
4 elaborated (PGY) to determine the antioxidant activity and to estimate the interaction
5 phenolics-proteins during 28 days of cold storage. Juice, yogurts and protein free
6 permeates were analyzed for phenolic composition. Yogurt fermentation modified the
7 anthocyanin profile of the initial PGJ, especially the content in cyanidin-3-*O*-glucoside.
8 During storage, individual anthocyanin content in PGY decreased but it did not modify
9 yogurt color. The analysis of permeates revealed that the degree of interaction phenol-
10 protein depends on the type of phenolic, being ellagic acid and dephinidin-3,5-*O*-
11 diglucoside the least bound phenolic compounds. The presence of PGJ in yogurt
12 enhanced radical scavenging performance, whereas all the observed ferric reducing
13 power ability of PGY was strictly due to the PGJ present. The 84.73% of total
14 anthocyanins remained bound to proteins at 1st day of storage and 90.06% after 28
15 days of cold storage revealing the high affinity of anthocyanins for milk proteins.

16 **KEY WORDS:** pomegranate, fermented milk, phenolic compounds, protein-polyphenol
17 interaction

18 **INTRODUCTION**

19 The pomegranate (PG, *Punica granatum* L.), a fruit extended throughout the
20 Mediterranean region, in Southeast Asia, California and Arizona in USA is one of the
21 oldest known edible fruit tree species.[1] In terms of crop yield, although having a very
22 little area (2000ha), Spain ranks first (18.5 t/ha) followed by the USA (18.3 t/ha). Due
23 to its immense potential for health benefits, PG has achieved the title of “super-food”.[2]
24 The health effects of whole pomegranate, its juices and extracts, have been studied in
25 relation to a variety of chronic diseases.[3,4] The *in vitro* antioxidant activity has been
26 attributed to its high polyphenolic content, specifically punicalagins, punicalins, gallagic
27 acid, and ellagic acid.[5]

28 The edible part of the PG fruit (50%) consists of 40% arils (which are used to
29 obtain juice) and 10% seeds. PG juice polyphenols are: hydrolysable tannins (mainly
30 ellagitannins), anthocyanins, non-colored flavonoids and phenolic acids.[6] In arils juice,
31 anthocyanins are the major group of phenolics and are responsible for its red color.

32 PGs are eaten fresh, and also used to obtain juice (PGJ), grenadine syrup (a
33 reduced juice from fresh PG seeds), “anardana” (dried PG raisins) and extracts. Also,
34 PGJ and seeds are used to prepare toppings, sauces and dips for many types of food.[2]
35 PGJ concentrate is currently used in the production of commercial PG Greek style
36 yogurts, especially in the USA and UK. The attractive red color provided by PG depends
37 on pigment concentration and it is one of the most important quality factors in fruit
38 yogurts.

39 The binding of polyphenols to milk proteins has been suggested to reduce their
40 bioavailability and functionality and, thus, to reduce their antioxidant potential.[7,8]
41 There are two main groups of proteins in milk, usually defined depending on their

42 solubility at pH 4.6 at 25 °C: caseins, constituting about 80% of the total proteins in
43 milk, and whey proteins.[9] Due to its relatively high charge, caseins show a tendency to
44 associate with other proteins according to the hydrophobic character of the micelle.[8]
45 Furthermore, caseins are proline-rich proteins which in turns have a strong affinity for
46 the hydroxyl (-OH) group of phenolic compounds.[8] We previously developed a new
47 dairy product formulated with PGJ rich in phenolic compounds[10] but the degree of
48 interaction protein-phenols and its effect on the antioxidant properties were still
49 unsolved. Juices solely made from arils are typically characterized by low phenolic
50 contents with the predominance of anthocyanins, gallotannins, hydroxybenzoic acids,
51 hydroxycinnamic acids and di-hydroflavonols.[11] Anthocyanins have been associated
52 with the prevention of cardiovascular disease, obesity, and diabetes.[12] Anthocyanins
53 are the major group of phenolic compounds in pomegranate juice, so their stability as
54 well as their interaction with milk proteins during yogurt shelf-life is of great interest.
55 The specific functionality of phenolic compounds in dairy products is based on their
56 ability to interact with milk proteins. Furthermore, the protein-polyphenol interaction
57 is maximal at the isoelectric point of the protein,[13] i.e. when yogurt is produced.

58 The aim of this work was to determine the antioxidant activity of a yogurt rich
59 in PGJ made from arils during 28 days of refrigerated storage as well as to estimate the
60 interaction between the phenolic compounds presents in the PGJ and milk proteins
61 and its impact on the antioxidant activity of the yogurt.

62 **MATERIALS AND METHODS**

63 **Materials and chemicals**

64 Commercial starter cultures of *Streptococcus thermophilus* and *Lactobacillus*
65 *delbrueckii* subsp. *bulgaricus* (Ezal© MY900, Rhodia Food-Danisco A/S, Sassenage,

66 France) were used at the concentrations recommended by the suppliers. For all the
67 study the same batch of skim milk powder was used (34% protein, 52% lactose, 1% fat,
68 6.8% ash, 5.2% moisture) (Central Lechera Asturiana, CAPSA, Granada-Siero, Spain).

69 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), Folin-Ciocalteu reagent, gallic acid, iron
70 (III) chloride, trichloroacetic acid (TCA), aluminium chloride, Trolox and formic acid
71 were from Sigma Chemical Company (Germany). Methanol of HPLC ultra-gradient
72 grade, HCl, potassium chloride, sodium acetate, dibasic potassium phosphate, sodium
73 nitrite (II), sodium hydroxide, sodium carbonate and dibasic sodium phosphate were
74 from Merck (Darmstadt, Germany). Potassium hexacyanoferrate was from Fluka
75 BioChemika (Germany). Ellagic acid was from Tocris Bioscience (Ellisville, MO, USA) and
76 pure punicalagins were from Chengdu Biopurity Phytochemicals Ltd. (Sischuan, China).
77 Cyanidin-3,5-*O*-diglucoside and -glucoside, pelargonidin-3-*O*-glucoside and delphinidin-
78 3-*O*-glucoside, were from Extrasynthese (Genay, France). Acetonitrile for HPLC-DAD
79 and LC/MS (Gradient grade) was from Merck (Darmstadt, Germany). LC grade water,
80 prepared by using an HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), was
81 additionally filtered through a 0.22 µm membrane filter immediately before use.

82 **Plant material**

83 Mature PG fruit cultivar 'Mollar de Elche', with no visible external cuts or
84 spoilage was purchased from a local market. The PG fruits were peeled manually and
85 the arils were introduced in a blender Ju2000 Vitae (Moulinex, Barcelona, Spain) to
86 obtain PGJ. PGJ was filtered through cotton gauze to remove particles and was kept
87 frozen until use.

88 **Yogurt manufacture**

89 Set-type yogurt was produced following the manufacture method developed by
90 Trigueros et al.,[10]. Briefly, skim milk powder (SMP) was reconstituted with deionised
91 water at 15 % w/v total solids to serve as control. SMP was reconstituted at 25% w/v
92 total solids to be further completed with PGJ to have a final solid content of 15% w/v
93 after pasteurization. PGJ, 15% reconstituted skim milk (RSM) and 25% RSM were
94 separately pasteurized at 80 ^{o}C for 30 min. After cooling to 43 ^{o}C, PGJ was added to the
95 RSM to a final concentration of 40 % v/v. At this point the starter culture was added
96 and the inoculated mix was incubated at 43 ^{o}C for 4h, and then cooled and stored at 4
97 ^{o}C for 28 days.

98 **Colorimetric analysis**

99 The CIE LAB color space of yogurts was studied, the following color coordinates
100 were determined: lightness (L*), redness (a*, +/- red-green), and yellowness (b*, +/-
101 yellow-blue). Color determinations were made at 12 ± 2 ^{o}C by means of a Minolta CM-
102 2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer, with a liquid accessory
103 CR-A70 (Minolta Camera Co., Osaka, Japan), with illuminant D_{65} and an observer of
104 10°.

105 **Preparation of the free-protein phase (permeate)**

106 In order to quantitatively estimate the interaction of phenolic compounds with
107 proteins, a protein free phase (permeate) was obtained with a laboratory scale
108 ultrafiltration (UF) cartridge (Amicon® Ultra-15; Merck Millipore Ltd., Ireland), with a
109 nominal cut-off of 10,000 Da and a nominal area of 7.6 cm^2 was used. To remove
110 caseins 30 g of yogurt were centrifuged at 7,000 rpm for 30 min, at to 4 ^{o}C. The
111 supernatant was ultrafiltrated through the cartridge at 5,500 rpm for 30 min, at 4 ^{o}C,
112 and permeate was collected for further analysis.

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113 **Extraction of phenolics from PJ and yogurt permeate**

114 PGJ and permeates (10 mL) were extracted with 30 mL of acidified methanol
115 (methanol containing 100 µL conc. HCl) for 30s at 12,000 rpm (IKA® T25 digital ULTRA-
116 TURRAX®, IKA® Werke Staufen, Germany). Then, the mixtures were centrifuged at
117 5,000 rpm for 10 min at 4°C. 5 mL of the extract was separated for determining
118 anthocyanin content. The remaining portions were evaporated to dryness using a
119 rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure (<100
120 mbar) at 50 ^{o}C and used for determining total phenolics, total flavonoids, antioxidant
121 activities and HPLC analysis.

122 **Extraction of phenolics from PJ yogurt**

123 Yogurt samples (20 g) were mixed with 30 mL of acidified methanol. Then the
124 mixture was left at 4 ^{o}C for overnight incubation. The mixture was filtered by using No.
125 1 Whatman filter paper and residue was washed with acidified methanol-HCl until it
126 became colorless.[14] Ten milliliters of the extract were separated for determining
127 anthocyanin content. The remaining portions were concentrated as previously
128 described and used for determining total phenolics, total flavonoids, antioxidant
129 activities and HPLC analysis.

130 **HPLC-DAD analysis (non-anthocyanin phenolics)**

131 Phenolic compounds were analyzed by high performance liquid
132 chromatography coupled with a diode array detector (HPLC-DAD) as previously
133 described by Benavente-García et al.[15] Samples (20 µL) were injected into a Hewlett-
134 Packard HPLC series 1200 instrument (Woldbronn, Germany) equipped with a C18
135 Teknokroma column (Mediterranean sea_{18} 25x0.4 cm, Teknokroma, Barcelona, Spain)
136 and detected by absorbance at 280 and 360 nm. UV spectra of individual peaks were

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137 recorded in the range of 200-600 nm. The separation columns were controlled
138 thermostatically at 35 ^{o}C. Phenolic compounds were analyzed in standard and sample
139 solutions using a gradient elution at 1 mL/min with the following gradient program (0-
140 20 min 95-75% A, 20-40 min 75-50% A, 40-45 min 50-95% A) with 4.5% formic acid in
141 water (v/v) as solvent A and 100% of acetonitrile as solvent B. Peaks were identified by
142 comparing retention times and photodiode array spectra with analytical standards.
143 The phenolic compounds were quantified by the absorbance of their corresponding
144 peaks with calibration curves of standard compounds. For this purpose, stock solutions
145 were diluted to concentrations of 10-160 mg/L (ellagic acid) and 25-800 mg/L
146 (punicalagins isomers).

147 **LC-PDA/MS analysis (anthocyanins)**

148 Identification of phenolic compounds was carried out using an ACQUITY Ultra
149 Performance LC^{TM} system (UPLC^{TM}) with binary solvent manager and photo diode array
150 (PDA) detector (Waters Corporation, Milford, MA, USA) and a Micromass Q-TOF Micro
151 mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization
152 (ESI) source operating in negative and positive mode. Separations of individual
153 phenolic compounds were carried out using a UPLC BEH C18 column (1.7 µm, 2.1 x 100
154 mm; Waters Corporation) at 30 ^{o}C. Samples (10 µL juice and 2 µL yogurt and permeate
155 extracts) were injected and elution completed in 20 min with a sequence of linear
156 gradients and isocratic flow rates of 0.45 mL/min. The mobile phase was composed of
157 solvent A (4.5% formic acid in water, v/v) and solvent B (100% of acetonitrile). The
158 programme began with isocratic elution with 99% A (0-1 min), and then a linear
159 gradient was used until 15 min, lowering A to 75%, and then 2.5 min for 0% of solvent
160 A and 2.5 min, returned to the initial composition (99% A) and then held constant to

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161 re-equilibrate the column. Analysis was carried out using full-scan, data-dependent MS
162 scanning from *m/z* 100 to 1500. The mass tolerance was 0.001 Da and the resolution
163 was 5,000. Leucine enkephalin was used as the internal reference compound during
164 ESI-MS accurate mass experiments and was permanently introduced via the LockSpray
165 channel using a Hamilton pump. The lock mass correction was ±1,000 for Mass
166 Window. All TOF-MS chromatograms are displayed as base peak intensity (BPI)
167 chromatograms and scaled to 12,400 counts per second (cps) (=100%). The effluent
168 was led directly to an electrospray source with a source block temperature of 130 ^{o}C,
169 desolvation temperature of 350 ^{o}C, capillary voltage of 2.5 kV and cone voltage of 30
170 V. Nitrogen gas used as desolvation gas at a flow rate of 300 L/h.

171 The characterisation of the single components was carried out via the retention
172 time and the accurate molecular masses. Each compound was optimized to its
173 estimated molecular mass in the negative and positive mode before and after
174 fragmentation. The data obtained from LC/MS were subsequently entered into the
175 MassLynx 4.0 ChromaLynxTM Application Manager software. Based on these data, the
176 software is able to scan different samples for the characterised substances.

177 For quantification of anthocyanin compounds spectra were measured over the
178 range 200-600 nm in steps of 2 nm. The run was monitored at 520 nm. Calibration
179 curves at concentration ranging from 0.05 to 5 mg/L (r^2 ≤ 0.9997) were made from
180 cyanidin-3,5-*O*-diglucoside and -glucoside, pelargonidin-3-*O*-glucoside and delphinidin-
181 3-*O*-glucoside. Delphinidin-3,5-*O*-diglucoside was expressed as delphinidin-3-*O*-
182 glucoside, pelargonidin-3,5-*O*-diglucoside as pelargonidin-3-*O*-glucoside and cyanidin-
183 pentoside as cyanidin-3-*O*-glucoside. All determinations were done in triplicate.
184 Results were expressed as milligrams per L.

185 **Determination of total phenol content, total flavonoid content and total anthocyanin**
186 **content by colorimetric methods**

187 The total phenol content (TPC) in the yogurts, PGJ, and permeates was
188 determined using the Folin-Ciocalteu's reagent.[16] Volumes of 0.3 mL of the methanolic
189 extracts were introduced into test tubes followed by 2.5 mL of Folin-Ciocalteu's
190 reagent (diluted 10 times with water) and 2 mL of sodium carbonate (7.5% w/v). The
191 tubes were vortexed and incubated at 50 ^{o}C for 5 min. Absorption at 760 nm was
192 measured with a HP 8451 spectrophotometer (Hewlet Packard, Cambridge, UK) and
193 compared to a gallic acid calibration curve. The results were expressed as mg gallic acid
194 equivalents (GAE)/g sample. Samples were analyzed in triplicate.

195 For total flavonoid content (TFC) the method based on Blasa et al.,[17] with some
196 modifications was used. One milliliter of the methanolic extracts of yogurts, PGJ, and
197 permeates was mixed with 0.3 mL $NaNO_2$ (5%), and 0.3 mL $AlCl_3$ (10%) were added
198 after 5 min. Samples were mixed and after 6 min, were neutralised with 2 mL NaOH
199 solution (1 M). Absorbance was read at 510 nm and the quantification was carried out
200 using a calibration curve. Different concentrations of rutin (8.5 - 170 µg/mL) were used
201 for calibration. The results were expressed in mg rutin equivalents (RE)/100 g of
202 sample as mean of three replicates.

203 Total anthocyanin content (TAC) in the yogurts and PGJ were determined by
204 the pH differential method described by Wrolstad[18] using a UV-vis HP 8451
205 spectrophotometer. Absorbance of the samples in 0.2 N potassium chloride buffer (pH
206 1.0) and 1 M sodium acetate buffer (pH 4.5) were measured at 520 and 700 nm.
207 Anthocyanin content was determined using the equation: $A = (A_{\lambda 520} - A_{\lambda 700})_{pH\ 1.0} - (A_{\lambda 520}$

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208 - $A_{\lambda 700})_{pH\ 4.5}$ with a molar extinction coefficient of 29,600. Results were expressed as mg
209 of cyanindin-3-glucoside equivalent/L of sample as mean of three replicates.

210 **Quantification of antioxidant activity by (i) DPPH$^{\bullet}$ radical scavenging method and (ii)**
211 **ferric reducing antioxidant power assay**

212 Free radical scavenging activities of the PGJ, yogurts, and permeates samples
213 were determined by DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) assay with minor
214 modifications as described by Brand-Williams et al.[19] A 0.1 mM solution of DPPH$^{\bullet}$ in
215 ethanol was prepared. A volume of 0.1 mL of various concentrations of the PGJ and
216 yogurt methanolic extracts diluted in methanol was added to 2.9 mL of DPPH$^{\bullet}$ solution.
217 The mixtures were well shaken in a Vortex (2,500 rpm) for 1 min and then placed in a
218 dark room. The decrease in absorbance at 517 nm was determined with a HP 8451
219 spectrophotometer after 1 h for all samples. Methanol was used to zero the
220 spectrophotometer. Absorbance of the radical without extract was used as control.
221 The amount of sample necessary to decrease the absorbance of DPPH$^{\bullet}$ by 50% (IC_{50})
222 was calculated graphically. The inhibition percentage of the DPPH$^{\bullet}$ was calculated from
223 the following equation: $\%I = [(A_C - A_S) / A_C] \times 100$. Where I = DPPH$^{\bullet}$ inhibition %; A_C =
224 absorbance of control sample (t = 0 h); A_S = absorbance of a tested sample at the end
225 of the reaction (t = 1 h).

226 The ferric reducing antioxidant power (FRAP) of PGJ, yogurts, and permeates
227 was determined by using the potassium ferricyanide-ferric chloride method.[20] One mL
228 of the PGJ, yogurt, and permeate methanolic extracts of four different concentrations
229 (2.5, 5.0, 7.5 and 10.0 g/100 mL) was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6)
230 and 2.5 mL potassium ferricyanide (1%). The mixtures were incubated at 50 ^{o}C for 20
231 min, after which 2.5 mL trichloroacetic acid (10%) was added. An aliquot of the mixture

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232 (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 0.1% $FeCl_3$. The
233 absorbance at 700 nm was measured after allowing the solution to stand for 30 min.
234 The FRAP of a sample is estimated in terms of Trolox equivalent antioxidant capacity
235 (TEAC) in mmol Trolox/L. Each essay was carried out in triplicate.

236 **Statistical analysis**

237 Statistical analysis and comparison among means were carried out using the statistical
238 package SPSS 20.0 (IBM SPSS Statistics, Chicago, IL, USA). ANOVA test was used to
239 evaluate factors: sample type (pomegranate yogurts and permeates) and storage time
240 (days 1, 14, 28). Tukey's pair wise comparisons test was used for means comparison
241 (95% confidence level). The whole experiment was independently run three times.

242

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243 **RESULTS AND DISCUSSION**

244 **Individual phenolic compounds content from pomegranate juice, yogurts, and**
245 **permeates**

246 Pomegranate anthocyanins were characterised by comparison of their UV–Vis
247 spectra (λ_{max}), retention times (R_t) and mass spectra (MS) before and after
248 fragmentation (MS/MS) with standards, if available, and published data. Figure 1
249 shows a typical UPLC chromatogram of pomegranate juice (A), pomegranate yogurt (B)
250 and yogurt permeate (C) anthocyanins recorded at 520 nm. The retention times, UV-
251 Vis and mass spectral characteristics as well as peak assignments for all compounds are
252 specified in Table 1.

253 The anthocyanin profiles of the pomegranate juice (PGJ) and pomegranate
254 yogurts (PGY) were similar; however, the amount of individual compounds differed. A
255 total of 7 anthocyanins were detected, 3 cyanidins and 2 delphinidins and 2

256 pelargonidins. Each of these anthocyanins were associated by monoglucoside or
257 diglucoside. The anthocyanins revealed the typical mass spectrometric behavior in
258 ESI(+) experiments, i.e. they showed M^+ ions in the MS experiments and the sequential
259 loss of their saccharide moieties, releasing the aglycones in the MS/MS experiments
260 (Table 1). These results agreed quite well with recently published data.[21,22] In
261 accordance with our results, previous analysis of juices produced from arils alone[21,23,24]
262 reported that anthocyanins were identified as the most abundant phenolic
263 compounds; other phenolics including ellagic acid and punicalagins were also
264 present.[24] Ellagic acid (EA) has exhibited both antioxidant[25] and anticarcinogenic[26]
265 properties together that it combines with proteins to form insoluble sediments.[27] EA
266 content was 21.17 mg/L in PGJ, 8.58 in PGY at 1st day and 11.75 mg/L in PGY at 28th
267 day. Our findings for EA in PGJ were in the range detected by Mena et al.,[28] (3.6 - 152.8
268 mg/L) and Gil et al.,[23] (33.2 mg/L) and slightly higher than those reported by Qu et al.,[24]
269 (2.1 - 15.3 mg/L) in juices made solely from arils. Regarding punicalagins (α + β)
270 content in PGJ was 46.35 mg/L, 31.90 mg/L in PGY at 1st day and 31.37 mg/L in PGY at
271 28th day of storage, being the content for all samples tested higher in the β anomer
272 (data not shown) which is typical of non-commercial juices.[28] In this sense our results
273 are in accordance to those reported by Mena et al.,[28] (α + β, 2.0 - 44.1 mg/L) and
274 higher than the amounts reported by Gil et al.,[23] (22.8 mg/L) and Qu et al.,[24] (4.1 - 22.8
275 mg/L). Many authors attribute the high antioxidant capacity of pomegranate juice to
276 hydrolysable tannins including punicalagins.[23,29] In permeates EA remained stable
277 showing values of 9.95 mg/L at the 1st day of storage and 10.87 mg/L at the 28th day of
278 the storage meaning that EA did not bind to milk proteins. However, no punicalagin
279 content was detected in yogurt permeates indicating a high affinity to milk proteins.

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280 Hence, only EA remained in free form in yogurt. The increase of EA during 28th days of
281 storage PGY and permeate is the result of the transformation of ellagitannins to ellagic
282 acid. Ellagic acid are transformed by human gut bacteria to the dibenzopyranone type
283 urolithins that are much better absorbed because of the higher lipophilicity of the
284 resulting components compared to intact form of punicalagins and ellagitannins.[30]

285 Table 2 shows the content of anthocyanins previously identified in
286 pomegranate juice, yogurts and permeates. Anthocyanins have been associated with
287 prevention of cardiovascular disease, obesity, and diabetes[31] and they are phenolic
288 compounds responsible for pomegranate juice color. In contrast to the other phenolic
289 compounds identified, PGJ was extremely rich in anthocyanins (641.48 mg/L). Similar
290 to our data, Fischer et al.,[21] reported an anthocyanin content of 558 mg/L in a
291 handmade juice from arils. Cyanidin-3-*O*-glucoside was the predominant anthocyanin
292 in juice; that predominance is characteristic for 'Mollar de Elche' varieties.[32,33] In
293 yogurts the relative quantitative distribution of the individual anthocyanins was slightly
294 different from PGJ. In yogurt samples the predominant anthocyanin was cyanidin-3,5-
295 *O*-glucoside followed by cyanidin-3-*O*-glucoside at all days analysed. In other PGJ
296 fermentation processes it has been studied losses up to 61% in the anthocyanin
297 content, mainly occurred during the initial fermentation. The structure influenced the
298 stability in the way that monoglucosides were less stable than diglucosides.[32] In the
299 present study both cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside were the
300 most affected anthocyanins in the fermentation process whereas cyanidin-3,5-*O*-
301 diglucoside was the most stable one. A similar trend has also been displayed in the
302 pomegranate winemaking process described by Mena et al.[32] Additionally the yoghurt
303 polymer network and gel matrix might have protected the polyphenols to some

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304 extent, through reducing the oxidation rates of these sensitive components.[34] During
305 the storage period significant losses in the individual anthocyanin content were
306 observed especially at the end of the storage (Figure 2). Degradation of anthocyanins
307 has been widely studied and it is well known that most anthocyanin follow a first-order
308 reaction kinetics[35-37] and even bacterial culture could affect the anthocyanin content
309 during storage probably associated with the production of antimicrobial compounds by
310 the starter culture.[38] Results from permeates revealed that most of the anthocyanins
311 remained bound to milk proteins; cyanidin-3,5-*O*-diglucoside, the main anthocyanin
312 present in the yogurts, was not detected in the permeate, neither delphinidin-3-*O*-
313 glucoside and cyanidin-pentoside did it. Delphinidin-3,5-*O*-diglucoside was found to be
314 the most stable anthocyanin and showed the lowest interaction rate with milk
315 proteins. The 84.73% of total anthocyanins remained bound to proteins at 1st day of
316 storage and 90.06% after 28 days of cold storage. Indeed, retentates (proteic phase)
317 had a more pronounced reddish color than their corresponding permeates. That could
318 be explained due to the monoglucosides of flavonoids showed stronger binding
319 affinities with milk proteins than their polyglucoside forms.[39] It has been demonstrated
320 that antioxidant activity of polyphenols decreases as interactions of milk proteins-
321 polyphenol complexes increase.[39] As far as we know no previous studies have been
322 conducted on the extent of interaction between individual anthocyanins and milk
323 proteins so results from this study would provide a first overview about the degree of
324 the interaction anthocyanin-milk protein giving knowledge about which anthocyanins
325 are available to exert the beneficial action. On the other hand, the interactions
326 between other flavonoids such as tea catechins have widely studied[7,8,40] and moreover
327 the effect of such interaction on antioxidant activity.[41] Arts et al.,[41] found that the

328 antioxidant activity of tea components was masked due to the addition of proteins, i.e.
329 assessing separately the antioxidant activity of flavonoid and protein yields higher
330 values than the antioxidant activity of the mixture tea-milk. Additionally they proved
331 that masking not only depends on the type of protein but also on the type of flavonoid;
332 proteins which contains more proline groups and flavonoids such as epigallocatechin
333 gallate (EGCG) and epicatechin gallate in green tea and gallic acid in black tea are
334 responsible of the masking. However, the interaction protein-polyphenol could be
335 both reversible and irreversible depending on pH, temperature, and protein and
336 flavonoid concentration.[42] Nevertheless, new findings have pointed out to a protective
337 role of milk as a carrier of bioactive molecules such as flavonoids arguing that binding
338 of flavonoids such as EGCG to the casein micelles did not affect the bioefficacy of
339 EGCG.[43]

340 **Total phenol content, total flavonoid content and total anthocyanin content**

341 Figure 3 shows total phenol content (TPC), total flavonoid content (TFC) and
342 total anthocyanin content (TAC) of pomegranate juice, pomegranate yogurts,
343 pomegranate yogurt permeates, and control yogurts. As expected, among the assayed
344 samples the higher phenolic content was observed in the PGJ (707.25 mg GAE/L). We
345 found lower phenolic substances than did previous reports[10,28] for the same
346 pomegranate cultivar group. Both aril composition and differing textural properties of
347 fruit tissues may influence the transfer of phenolic compounds into the juices upon
348 processing.[11] PGY contained 40% of juice and presented 241.44 mg GAE/L of total
349 phenolic substances, which means an 85.35% of the theoretically expected. On the
350 first day of storage the TPC in JY permeates was 111.92 mg GAE/L which means that
351 nearly 54% of the total phenolic substances remained in the proteic phase interacting

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352 with milk proteins. At the end of the storage period this percentage decreased
353 (40.50%) being the TPC in PGY and yogurt permeates 142.60 mg GAE/L and 111.92 mg
354 GAE/L, respectively. PGY exhibited the most remarkable decrease (40.94%) in phenolic
355 concentration ($p<0.05$) during the storage period followed by yogurt permeates
356 (24.19%), being CY the most stable (4.32%) sample. Other studies showed the same
357 tendency in phenolic content reducing in enriched yogurts with phenolic compounds
358 stored during 14 days[14] and 7 days[44]. The stability of pigments and phenolics in yogurts
359 is affected by storage temperature, pH, phenolic content, fat[38,44] and the type of
360 bacterial culture used.[38] Taking into account the TFC in the juice (75.50 mg RE/100 g)
361 the observed in JY was higher than expected (34.13 mg RE/100 g). Permeates retained
362 the 65.78% and 90% of the flavonoids on the 1st day and 28th day, respectively. So we
363 have estimated by colorimetric methods that flavonoids have less affinity to proteins
364 than other phenolic compounds. Interestingly, although yogurt is not being considered
365 as a significant source of phenolic compounds[13] CY presented 39.08 mg GAE/L and 3.78
366 mg RE/100 g on the 1st day of storage for TPC and TFC. During the storage period TFC
367 decreased 39.93%, 17.82% and 23.28% in PGY, yogurt permeates and CY, respectively
368 (Figure 3) although such decrease was only statistically significant for PGY. The
369 phenolic and flavonoid contents can be used as important indicators of antioxidant
370 capacity in the screening of natural sources of antioxidants.[45]

371 PGJ presented a total anthocyanin content of 126.04 mg cyanidin-3-glucoside
372 equivalents/L. Quantitatively, our results are higher than those reported by Elfalleh et
373 al.,[44] (39.19 mg CGE/L) and Ozgen et al.,[47] (60.0 mg CGE/L) as analysed by the same
374 method, but lower than our own LC-MS results an those of by Gil et al.,[23] (306.0 mg/L)
375 as assessed by chromatographic method. Chromatographic methods and results are

376 considered more accurate than colorimetric methods given their limitations and
377 expression under an individual representative compound. However colorimetric
378 methods may be still of interest where more sophisticated chromatographic methods
379 are not available.

380 Due to naturally neither milk nor control yogurts do not provide anthocyanins,
381 we found that such content was relatively high in PGY during the storage period (56.91
382 and 57.60 mg cyanidin-3-glucoside equivalents/L at 1st and 28th day, respectively).
383 Anthocyanin concentrations of PGY as assessed by colorimetric methods were not
384 affected ($p>0.05$) by storage time which is not correlated with the previous HPLC
385 results. But these results were in accordance with those obtained from color
386 determinations in yogurt (Table 3). During the storage period the recorded a^* and b^*
387 values showed significant variations; redness (a^*) increased and yellowness (b^*)
388 decreased meaning that the reddish color was reinforced. Although not statistically,
389 lightness (L^*) decreased during storage also a clear sign of pigment stabilization. This
390 stabilization could be due to intermolecular/intramolecular, copigmentation, and self-
391 association reactions.[42] Furthermore, Jing and Giusti[45] found that anthocyanins were
392 more stable in milk matrices than in a phosphate buffer solution used as control. In the
393 study carried out by Srivastava et al.,[46] a relatively superior content of malvidin
394 glucosides was observed in a stored blueberry extract. In yogurt permeates non
395 anthocyanin content was detected by colorimetric quantitative methods.

396 **Antioxidant activity in pomegranate juice, yogurts and permeates**

397 In this study the *in vitro* antioxidant activity (AA) of pomegranate juice and
398 yogurts and permeates during 28 days of storage was measured by two different
399 analytical methods: $DPPH^•$ and FRAP (results shown in Table 4a). The free radical

400 scavenging activity of the samples were reported as the amount of the juice, yogurt or
401 permeate (g/100 mL) required to scavenge 50% of DPPH$^{\bullet}$ (IC_{50}), and the lower IC_{50}
402 values the higher the antioxidant power. PGY displayed the highest AA in comparison
403 to the other samples. PGJ showed a poor free radical scavenging activity by DPPH•
404 method, similar to the reported in a previous own study, 7.47.[10] Considering the
405 results obtained for CY it is possible that a synergistic action is taken place when the
406 pomegranate yogurt is produced. Indeed we analyzed the unfermented mix of milk
407 and PGJ (data not presented) resulting in a IC_{50} value of 4.72. During the storage time
408 enriched yogurts slightly increased the radical scavenging activity, and the same
409 pattern was found in the study carried out by Jiménez et al.[47] Nonetheless, at the end
410 of the storage TPC and TFC decreased significantly (Figure 3) being anthocyanins (TAC)
411 the phenolic compound which remained stable. Hence, is it possible that the radical
412 scavenging activity of the PGY could be done by the anthocyanins. Oppositely, studies
413 carried out in yogurts fortified in phenolic extracts showed a tendency in decreasing
414 the AA during the storage period.[14]
415 According to the FRAP values, a concentration-dependent ferric reducing
416 capacity was found for all the samples analyzed. PGJ showed by far the highest
417 (p<0.05) ferric reducing capacity in terms of Trolox concentrations. In juice FRAP was
418 strongly correlated with TPC and TAC (Table 5). In PGY at day 1 of storage results from
419 FRAP assay corresponded to nearly 40% of those of PGJ, so due to the low AA of CY
420 detected by this method the AA of PGY was done by PGJ. In Table 4b we could easily
421 intuit how different behave the same potentially antioxidant sample in the face of
422 different antioxidant methods. Only the ferric reducing capacity was affected (p<0.05)
423 by the storage time in PGY. At the end of the storage no difference were found

424 between PGY and permeates. Furthermore, the AA in CY was correlated with TPC
425 (Table 5).

426 A point worth mentioning is the poor correlation between phenolic compounds
427 and antioxidant activity in PGY in comparison to juice and permeates (Table 5).
428 Permeates and juices are free of proteins so phenolics are mainly in free form.
429 However yogurt is a complex matrix where several interactions could occur so it is not
430 clear whether the antioxidant capacity of the PGY is given by phenolics alone or by a
431 multifactorial cause. This fact may mean that other components naturally present in
432 PGJ, such as ascorbic acid or vitamin E, need to be investigated for their contribution
433 to the AA.[12]

434 In conclusion, yogurts enriched in pomegrante juice are especially rich in anthocyanins,
435 also other phenolic compounds such ellagic acid and punicalagins are also present at
436 significant levels. Fermentation process affects the anthocyanin profile; the content in
437 cyanidin-3-*O*-glucoside, the main anthocyanin present in the juice, is significantly
438 reduced whereas cyanidin-3,5-*O*-diglucoside remains much more stable pointing out
439 that structure (mono or diglucoside form) affects the anthocyanin stability. During the
440 storage period the individual anthocyanin content is diminished which is not detected
441 in color measurements of yogurt. Results from permeates reveal that the interaction
442 of phenolic compounds with milk proteins occurs at different level depending on the
443 type of phenolic: ellagic acid and delphinidin-3,5-*O*-diglucoside show the lowest
444 affinity to bind proteins.

445 Considerable variation in antioxidant activity is observed depending on the antioxidant
446 assay. Pomegranate juice shows the strongest ferric reducing capacity whereas
447 pomegranate yogurts display the highest radical scavenging activity. The presence of

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448 PGJ in yogurt enhanced radical scavenging performance, whereas all the observed
449 ferric reducing power ability of PGY was strictly due to the PGJ present. Pomegranate
450 yogurt can be considered a good antioxidant dairy product, and also during the storage
451 period in terms of scavenging activity. Permeates in general had a poor antioxidant
452 activity. The interaction of PG phenolics with milk proteins does not affect the
453 antioxidant activity of yogurts.
454

455 **Abbreviations Used**

456 PG, pomegranate; PGJ, pomegranate juice; PGY, pomegranate yogurt; CY, control
457 yogurt; HPLC, high performance liquid chromatography; *L**, lightness; *a**, redness; *b**,
458 yellowness; TPC, total phenol content; TFC, total flavonoid content; TAC, total
459 anthocyanin content; AA, antioxidant activity; DPPH, 2,2-diphenyl-1-picrylhydrazyl;
460 FRAP, ferric reducing antioxidant power.
461

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465

466 **References**

467 (1) Miguel, M. G.; Neves, M. A.; Antunes, M. D. Pomegranate (*Punica granatum* L.): a
468 medicinal plant with myriad biological properties - a short review. *J. Med. Plants Res*.
469 **2010**, *4*, 2836-2847.
470 (2) Teixeira da Silva, J. A.; Rana, T. S.; Narzary, D.; Verma, N.; Meshram, D. T.; Ranade,
471 S. A. Pomegranate biology and biotechnology: a review. *Sci. Hort*. **2013**, *160*, 85-107.

472 (3) Syed, D. N.; Afaq, F.; Mukhtar, H. Pomegranate derived products for cancer
473 chemoprevention. *Semin. Cancer Biol*. **2007**, *17*, 377-385.
474 (4) Wang, R.; Ding, Y.; Liu, R.; Xiang, L.; Du, L. Pomegranate: constituents, bioactivities
475 and pharmacokinetics. *Fruit, Veg. Cereal Sci. Biotech*. **2010**, *4*, 77-87.
476 (5) Johanningsmeier, S. D.; Harris, G.K. Pomegranate as a functional food and
477 nutraceutical source. *Annu. Rev. Food Sci*. **2011**, *2*, 181-201.
478 (6) Mena, P.; Calani, L.; Dall'Asta, C.; Galaverna, G.; García-Viguera, C.; Bruni, R.;
479 Crozier, A.; Del Rio, D. Rapid and comprehensive evaluation of (poly)phenolic
480 compounds in pomegranate (*Punica granatum* L.) juice by UHPLC-MS^n. *Molecules*.
481 **2012**, *17*, 14821-14840.
482 (7) Jöbstl, E.; Howse, J. R.; Fairclough, J. P. A.; Williamson, M. P. Noncovalent cross-
483 linking of casein by epigallocatechin gallate characterized by single molecule force
484 microscopy. *J. Agric. Food Chem*. **2006**, *54*, 4077-4081.
485 (8) Yuksel, Z.; Avci, E.; Erdem, Y. K. Characterization of binding interactions between
486 green tea flavonoids and milk proteins. *Food Chem*. **2010**, *121*, 450-456.
487 (9) Yazdi, S. R.; Corredig, M. Heating of milk alters the binding of curcumin to casein
488 micelles. A fluorescence spectroscopy study. *Food Chem*. **2012**, *132*, 1143-1149.
489 (10) Trigueros, L.; Viuda-Martos, M.; Pérez-Álvarez, J. A.; Sendra, E. Low fat set yoghurt
490 rich in pomegranate juice: a new antioxidant dairy product. *Milchwissenschaft*. **2012**,
491 *67*, 177-180.
492 (11) Fischer, U. A.; Jaksch, A. V.; Carle, R.; Kammerer, D. R. Influence of origin source,
493 different fruit tissue and juice extraction methods on anthocyanin, phenolic acid,
494 hydrolysable tannin and isolariciresinol contents of pomegranate (*Punica granatum* L.)
495 fruits and juices. *Eur. Food Res. Technol*. **2013**, *237*, 209-221.

496 (12) Viladomiu, M.; Hontecillas, R.; Lu, P.; Bassaganya-Riera, J. Preventive and
497 prophylactic mechanisms of action of pomegranate bioactive constituents. *Evid-Based*
498 *Compl. Alt.* **2013**, http://dx.doi.org/10.1155/2013/789764
499 (13) O'Connell, J. E.; Fox, P. F. Significance and applications of phenolic compounds in
500 the production and quality of milk and dairy products: a review. *Int. Dairy J*. **2001**, *11*,
501 103-120.
502 (14) Karaaslan, M.; Ozden, M.; Vardin, H.; Turkoglu, H. Phenolic fortification of yogurt
503 using grape and callus extracts. *LWT - Food Sci. Technol*. **2011**, *44*, 1065-1072.
504 (15) Benavente-García, O.; Castillo, J.; Lorente, J.; Ortuño, A.; Del Río, J. A. Antioxidant
505 activity of phenolics extracted from *Olea europea* L. leaves. *Food Chem*. **1999**, *68*, 457-
506 462.
507 (16) Singlenton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-
508 phosphotungstic acid reagents. *Am. J. Enol. Viticult*. **1965**, *16*, 144-158.
509 (17) Blasa, M.; Candiracci, M.; Accorsi, A.; Piacentini, P. M.; Albertini, M. C.; Piatti, E.
510 Raw Millefiori honey is packed full of antioxidants. *Food Chem*. **2005**, *97*, 217–222.
511 (18) Wrolstad, R. E. In *Color and pigments analyses in fruit products*. Agricultural
512 Experiment Station, Oregon State University, Corvallis, Oregon. Station Bulletin. 1976,
513 reprinted 1993, 624, 1-17.
514 (19) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to
515 evaluate antioxidant activity. *LWT – Food Sci. Technol*. **1995**, *28,* 25-30.
516 (20) Oyaizu, M. Studies on products of browning reactions: antioxidative activities of
517 products of browning reaction prepared from glucosamine. *Jpn. J. Nutr*. **1986***, 44*, 307-
518 315.

519 (21) Fischer U. A.; Carle R.; Kammerer, D. R. Identification and quantification of
520 phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and
521 differently produced juices by HPLC-DAD–ESI/MSn. *Food Chem*. **2011**, *127*, 807–821.
522 (22) Gil, M.; Garcia-Viguera, C.; Artés, F.; Tomás-Barberán, F. Changes in pomegranate
523 juice pigmentation during ripening. *J. Sci. Food Agr*. **1995**, *68*, 77–81.
524 (23) Gil, M. I.; Tomás-Barberán, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. A.
525 Antioxidant activity of pomegranate juice and its relationship with phenolic
526 composition and processing. *J. Agric. Food Chem*. **2000**, *48*, 4581-4589.
527 (24) Qu, W.; Breksa III, A. P.; Pan, Z.; Ma, H. Quantitative determination of major
528 polyphenol constituents in pomegranate products. *Food Chem*. **2012**, *132*, 1585-1591.
529 (25) Falsaperla, M.; Morgia, G.; Tartarone, A.; Ardito, R.; Romano, G. Support ellagic
530 acid therapy in patients with hormone refractory prostate cancer (HRPC) on standard
531 chemotherapy using vinorelbine and estramustine phosphate. *Eur.Urol*. **2005**, *47*, 449-
532 454.
533 (26) Hassoun, E. A.; Vodhanel, J.; Abushaban, A. The modulatory effects of ellagic acid
534 and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of
535 rats after subchronic exposure. *J. Biochem. Mol. Toxic*. **2004**, *18*, 196-203.
536 (27) Niino, H.; Sakane, I.; Okanoya, K.; Kuribayashi, S., Kinugasa, H. Determination of
537 mechanism of flock sediment formation in tea beverages. *J. Agric. Food Chem*. **2005**,
538 *53*, 3995-3999.
539 (28) Mena, P.; García-Viguera, C.; Navarro-Rico, J.; Moreno, D. A.; Bartual, J.; Saura, D.;
540 Martí, N. Phytochemical characterisation for industrial use of pomegranate (*Punica*
541 *granatum* L.) cultivars grown in Spain. *J. Sci. Food Agric*. **2011**, *91*, 1893-1906.

UNIVERSITAS Miguel Hernández

542 (29) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartfield, P.
543 W.; Riechel, T. L. High molecular weight plant polyphenolics (tannins) as biological
544 antioxidants. *J. Agric. Food Chem*. **1998**, *46*, 1887-1892.
545 (30) Bialońska D.; Ramnani P.; Kasimsetty S. G.; Muntha K. R.; Gibson G. R.; Ferreira D.
546 The influence of pomegranate by-product and punicalagins on selected groups of
547 human intestinal microbiota. *Int. J. Food Microbiol*. **2010**, *140*, 175-182.
548 (31) Jurenka, J. Therapeutic applications of pomegranate (*Punica granatum* L.): a
549 review. *Altern. Med. Rev*. **2008**. *13*, 128-144.
550 (32) Mena, P.; Gironés-Vilaplana, A.; Martí, N.; García-Viguera, C. Pomegranate varietal
551 wines: phytochemical composition and quality parameters. *Food Chem*. **2012**, *133*,
552 108-115.
553 (33) Pérez-Vicente, A.; Serrano, P.; Abellán, P.; García-Viguera, C. Influence of
554 packaging material on pomegranate juice color and bioactive compounds during
555 storage. *J. Sci. Food Agric*. **2004**, *84*, 639-644.
556 (34) Sun-Waterhouse, D., Zhou, J., Wadhwa, S. S. Drinking yoghurts with berry
557 polyphenols added before and after fermentation. *Food Control*. **2013**, *32*, 450-460.
558 (35) Giusti, M. M.; Wrolstad, R. E. Radish anthocyanin extract as a natural red colorant
559 for maraschino cherries. *J. Food Sci.* **1996**, *61*, 688-694.
560 (36) Iversen, C. K. Black currant nectar: effect of processing and storage on
561 anthocyanin and ascorbic acid content. *J. Food Sci*. **1999**. *64*, 37-41.
562 (37) Wang, W. D.; Xu, S. Y. Degradation kinetics of anthocyanins in blackberry juice and
563 concentrate. *J. Food Eng*. **2007**, *82*, 271-275.

564 (38) Ścibisz, I.; Ziarno, M.; Mitek, M.; Zaręba, D. Effect of probiotic cultures on the
565 stability of anthocyanins in blueberry yoghurts. *LWT - Food Sci. Technol*. **2012**, *49*, 208-
566 212.
567 (39) Xiao, J.; Mao, F.; Yang, F.; Zhao, Y.; Zhang, C.; Yamamoto, K. Interaction of dietary
568 polyphenols with bovine milk proteins: molecular structure-affinity relationship and
569 influencing bioactivity aspects. *Mol. Nutr. Food Res*. **2011**, *55*, 1637-1645.
570 (40) Kanakis, C. D.; Hasni, I.; Bourassa, P.; Tarantilis, P. A.; Polissiou, M. G.; Tajmir-Riahi,
571 H.-A. Milk *β*-lactoglobulin complexes with tea polyphenols. *Food Chem*. **2011**, *127*,
572 1046-1065.
573 (41) Arts, M. J. T. J.; Haenen, G. R. M. M.; Wilms, L. C.; Beetstra, S. A. J. N.; Heijnen, C.
574 G. M.; Voss, H.-P.; Bast, A. Interactions between flavonoids and proteins: effect on the
575 total antioxidant capacity. *J. Agric. Food Chem*. **2002**, *50*, 1184-1187.
576 (42) Luck, G.; Liao, H.; Murray, J.; Grimmer, H.; Warminski, E.; Williamson, M.; Lilley, E.
577 H.; Haslam, E. Polyphenols, astringency and proline-rich proteins. *Phytochemistry*.
578 **1994**, *37*, 357-371.
579 (43) Haratifar, S.; Meckling, K. A.; Corredig, M. Antiproliferative activity of tea catechins
580 associated with casein micelles, using HT29 colon cancer cells. *J. Dairy Sci*. **2014**, *97*,
581 672-678.
582 (44) Wallace, T. C.; Giusti, M. M. Determination of color, pigment, and phenolic
583 stability in yogurt systems colored with nonacylated anthocyanins from *Berberis*
584 *boliviana* L. as compared to other natural/synthetic colorants. *J. Food Sci*. **2008**, *73*,
585 C241-C248.
586 (45) Viuda-Martos, M.; Ruiz-Navajas, Y.; Fernández-López, J.; Sendra, E.; Sayas-Barberá,
587 E.; Pérez-Álvarez, J. A. Antioxidant properties of pomegranate (*Punica granatum* L.)

588 bagasses obtained as co-product in the juice extraction. *Food Res. Int*. **2011**, *44*, 1217-
589 1223.
590 (46) Elfalleh, W.; Tlili, N.; Nasri, N.; Yahia, Y.; Hannachi, H.; Chaira, N.; Ying, M.;
591 Ferchichi, A. Antioxidant capacities of phenolic compounds and tocopherols from
592 Tunisian pomegranate (*Punica granatum*) fruits. *J. Food Sci*. **2011**, *76*, C707-C713.
593 (47) Ozgen, M.; Durgaç, C.; Serçe, S.; Kaya, C. Chemical and antioxidant properties of
594 pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chem*.
595 **2008**, *111*, 703-706.
596 (48) Jing, P.; Giusti, M. M. Characterization of anthocyanin-rich waste from purple
597 corncobs (*Zea mays* L.) and its application to color milk. *J. Agric. Food Chem*. **2005**, *53*,
598 8775-8781.
599 (49) Srivastava, A.; Akoh, C. C.; Yi, W.; Fischer, J.; Krewer, G. Effects of storage
600 conditions on the biological activity of phenolic compounds of blueberry extract
601 packed in glass bottles. *J. Agric. Food Chem*. **2007**, *55*, 2705-2713.
602 (50) Jiménez, A. M.; Murcia, M. A.; Parras, P.; Martínez-Tomé, M. On the importance of
603 adequately choosing the ingredients of yoghurt and enriched milk for their antioxidant
604 activity. *International Journal of Food Science and Technology*. **2008**, *43*, 1464-1473.
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607 **Figure captions**

608 Figure 1. Chromatograms of anthocyanins in A) pomegranate juice, B) pomegranate
609 juice yogurt, C) permeate from pomegranate yogurt at 520 nm

610

611 Figure 2. Chromatograms of anthocyanins in pomegranate yogurts after 1 (A), 14 (B)
612 and 28 (C) days of cold storage

613

614 Figure 3. Total phenol content (TPC; mg GAE/L), total flavonoid content (TFC; mg
615 RE/100 g) and total anthocyanin content (TAC; mg cyanidin-3-glucoside/L) of
616 pomegranate juice and yogurts and permeates over 28 days of storage at 4 ^{o}C

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Tables

Table 1. LC/MS Analysis of Anthocyanin Compounds in Pomegranate Juices and Pomegranate Yogurt

Peak	Compound	R_t (min)	λ_{max} (nm)	$[M+H]^+$ (*m/z*)	MS/MS (*m/z*)
1	Delphinidin-3,5-*O*-diglucoside	5.70	277; 519	627	465/303/186
2	Cyanidin-3,5-*O*-diglucoside	6.82	277; 513	611	449/287/186
3	Delphinidin-3-*O*-glucoside	7.25	277; 522	465	303/186
4	Pelargonidin-3,5-*O*-diglucoside	7.99	274; 499	595	433/274/303/186
5	Cyanidin-3-*O*-glucoside	8.49	280; 516	449	287
6	Pelargonidin-3-*O*-glucoside	9.77	274; 503	433	271
7	Cyanidin-pentoside	10.80	277; 513	419	287

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Table 2. Content of Individual Anthocyanins (mg/L) in Pomegranate Juice, Yogurts, and Protein Free Permeates[b]

	Anthocyanins							
Sample[a]	Dp 3,5-diglc	Cy 3,5-diglc	Dp 3-glc	Pg 3,5-diglc	Cy 3-glc	Pg 3-glc	Cy-pent	Total anthocyanins
PGJ	78.41±0.78	190.03±1.88	62.85±0.65	21.55±0.25	221.00±2.20	64.65±0.65	3.00±0.10	641.48±1.25
PGY d1	61.80±0.60	162.70±1.60	49.35±0.45	16.45±0.15	125.65±1.25	24.45±0.25	1.75±0.05	442.15±3.05
PGY d14	59.85±0.55	149.65±1.45	43.45±0.45	15.75±0.15	110.40±1.10	23.05±0.25	1.55±0.05	403.70±0.10
PGY d28	31.40±0.30	103.10±1.00	33.10±0.30	9.85±0.25	80.60±0.80	16.35±0.15	1.25±0.05	275.65±0.85
Permeate d1	47.55±1.45	0.00±0.00	0.00±0.00	3.45±0.05	12.85±015	3.65±0.05	0.00±0.00	67.50±1.20
Permeate d28	16.85±1.15	0.00±0.00	0.00±0.00	1.25±0.05	7.25±0.15	2.05±0.05	0.00±0.00	27.40±0.90

[a] Dp-3,5-diglc, delphinidin-3-5-*O*-diglucoside; Cy-3,5-diglc, cyanidin-3,5-*O*-diglucoside; Dp-3-glc, delphinidin-3-*O*-glucoside; Pg-3,5-diglc, pelargonidin-3,5-*O*-diglucoside; Cy-3-glc, cyanidin-3-*O*-glucoside; Pg-3-glc, pelargonidin-3-*O*-glucoside; Cy-pent, cyanidin-pentoside; PGJ, pomegranate juice; PGY, pomegranate juice yogurt.

[b] Values expressed as means of duplicate ± standard error. Significant differences between values in the same column are indicated by different small letters ($p<0.05$) according to Tukey's multiple range test.

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Table 3. Color Characteristics of Yogurts during 28 Days of Cold Storage[a].

Type of yogurt	*L**	*a**	*b**
Control	84.61±1.16[b]	-2.73±0.06[a]	5.27±0.17[c]
PGY d1	66.65±0.41[a]	5.61±0.10[b]	4.93±0.11[b]
PGY d14	65.95±0.05[a]	5.78±0.02[b]	4.83±0.05[a,b]
PGY d28	65.24±0.52[a]	6.26±0.04[c]	4.56±0.04[a]

[a]Values expressed as means of triplicate ± standard error. Significant differences between values in the same column are indicated by different small letters ($p<0.05$) according to Tukey's multiple range test.

Table 4a. Antioxidant Activity of Pomegranate Juice and Yogurts and Protein Free Permeates During Storage at 4 ^{o}C Measured by the DPPH$^{\bullet}$ assay and the FRAP Method at Different Concentrations (A = 2.5 g/100 mL, B = 5 g/100 mL, C = 7.5 g/100 mL, D = 10 g/100 mL)[e]

Sample	Storage time (day)	DPPH$^{\bullet}$	FRAP			
		IC_{50}[a]	TEAC[b] (mM Trolox/L)			
			A	B	C	D
Juice		10.45	0.254 ± 0.006^{aD}	0.479 ± 0.001^{bE}	0.710 ± 0.011^{cD}	0.870 ± 0.011^{dD}
PGY[c]	1st	2.51	0.090 ± 0.003^{aC}	0.132 ± 0.002^{a,bD}	0.215 ± 0.036^{b,cC}	0.279 ± 0.030^{cC}
	28th	2.45	0.060 ± 0.002^{aB}	0.115 ± 0.001^{bC}	0.163 ± 0.002^{cB,C}	0.202 ± 0.009^{dB}
PGY permeate	1st	70.62	0.061 ± 0.001^{aB}	0.111 ± 0.001^{bC}	0.164 ± 0.001^{cB,C}	0.208 ± 0.004^{dB}
	28th	85.46	0.049 ± 0.001^{aB}	0.089 ± 0.003^{bB}	0.132 ± 0.001^{cB}	0.162 ± 0.002^{dB}
CY[d]	1st	5.38	0.023 ± 0.002^{aA}	0.036 ± 0.000^{bA}	0.048 ± 0.000^{cA}	0.062 ± 0.001^{dA}
	28th	6.96	0.022 ± 0.000^{aA}	0.028 ± 0.004^{a,bA}	0.044 ± 0.000^{b,cA}	0.054 ± 0.004^{cA}

[a] IC_{50}, concentration (g/100 mL) for a 50% of inhibition. [b] TEAC, Trolox equivalent antioxidant capacity. [c] PGY, pomegranate yogurt. [d] CY, control yogurt.
[e] Values expressed as means of duplicate ± standard error. Significant differences between values in the same line are indicated by different small letters ($p<0.05$) according to Tukey's multiple range test. Significant differences between values in the same column are indicated by different capital letters ($p<0.05$) according to Tukey's multiple range test.

Table 4b. Relative Antioxidant Activity of Pomegranate Juice and Yogurts and Protein Free Permeates

Sample	DPPH$^{\bullet}$	FRAP
Juice	++	++++
PGY	++++	++
PGY permeate	-	++
CY	+++	+

See Table 4a for abbreviations.

Table 5. Correlation Matrix between Antioxidant Capacity Methods and Total Phenol Content (TPC), Total Flavonoid Content (TFC), and Total Anthocyanin Content (TAC) in Pomegranate Juice, Pomegranate Yogurts, Protein Free Permeates and in Control Yogurts[d]

		TPC	TFC	TAC
PGJ[a]	DPPH•	0.98	0.10	1
	FRAP	0.99	0.45	0.92
PGY[b]	DPPH•	0.64	0.88	0.24
	FRAP	0.89	0.77	0.72
PGY permeates	DPPH•	0.90	0.99	---
	FRAP	0.99	0.98	---
CY[c]	DPPH•	0.57	0.01	---
	FRAP	0.84	0.42	---

[a]PGJ, pomegranate juice. [b]PGY, pomegranate yogurt. [c]CY, control yogurt.
[d]The *r* value of the correlation is given. All correlations were significant at $p<0.05$.

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Figure graphics

Figure 1

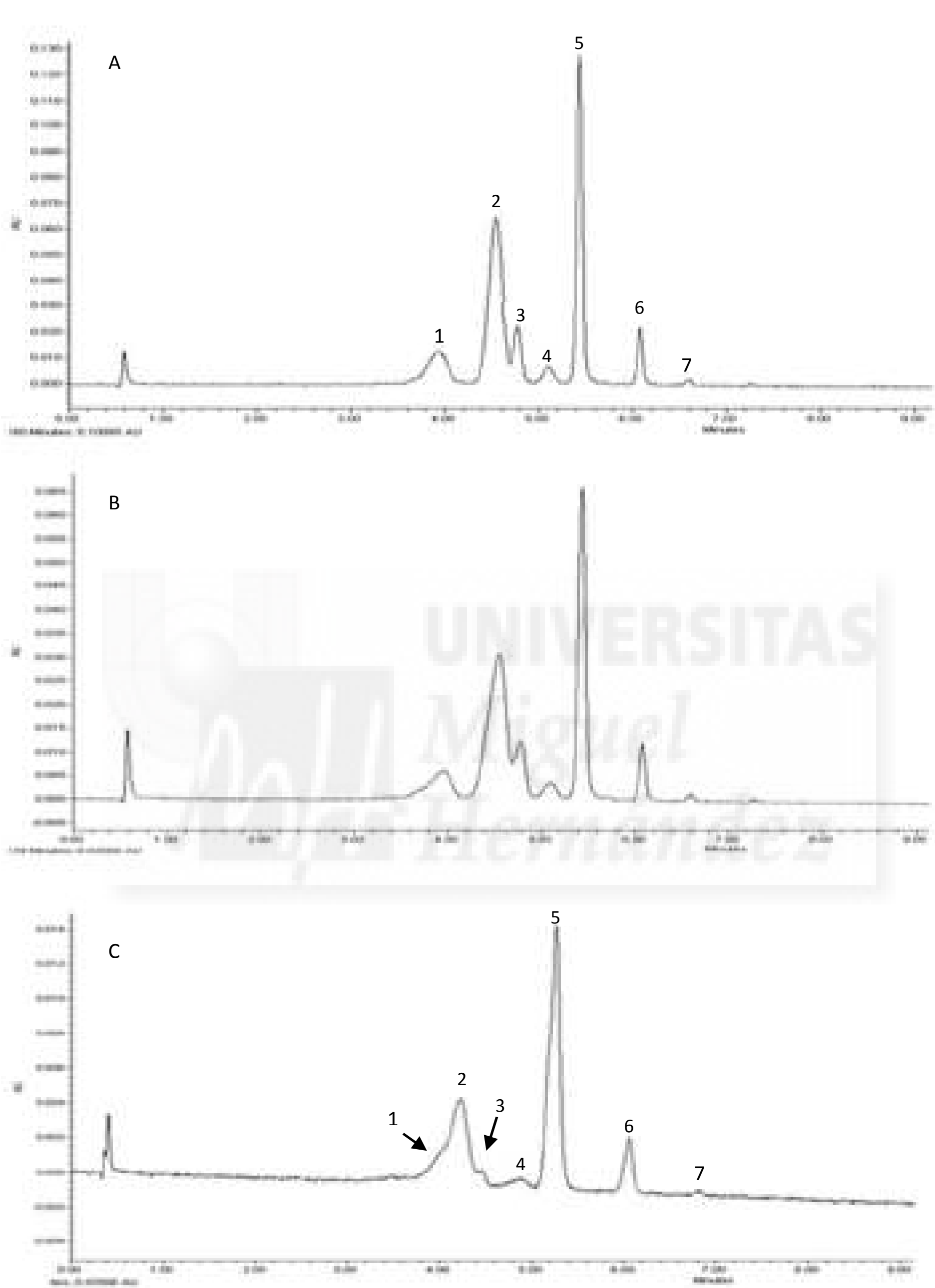


Figure 2

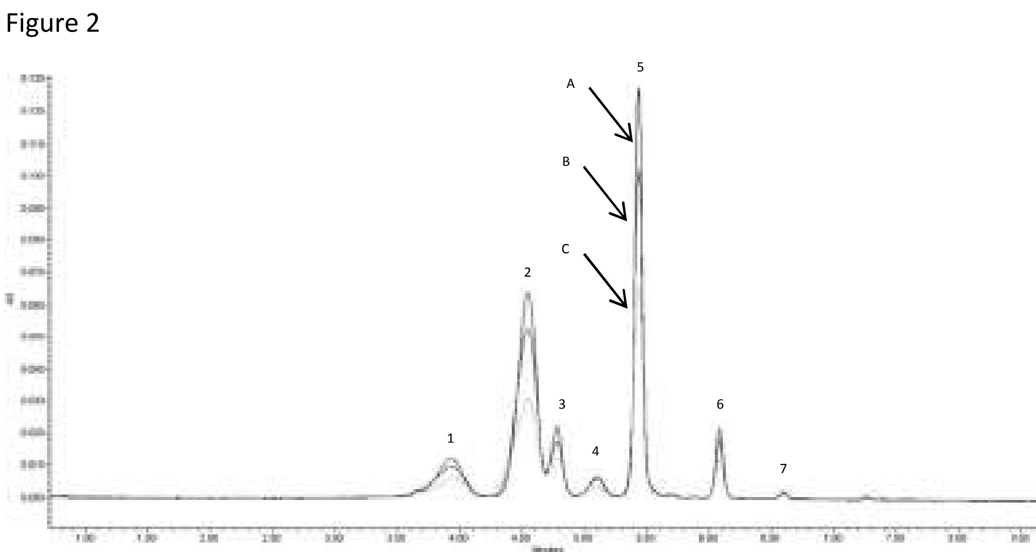
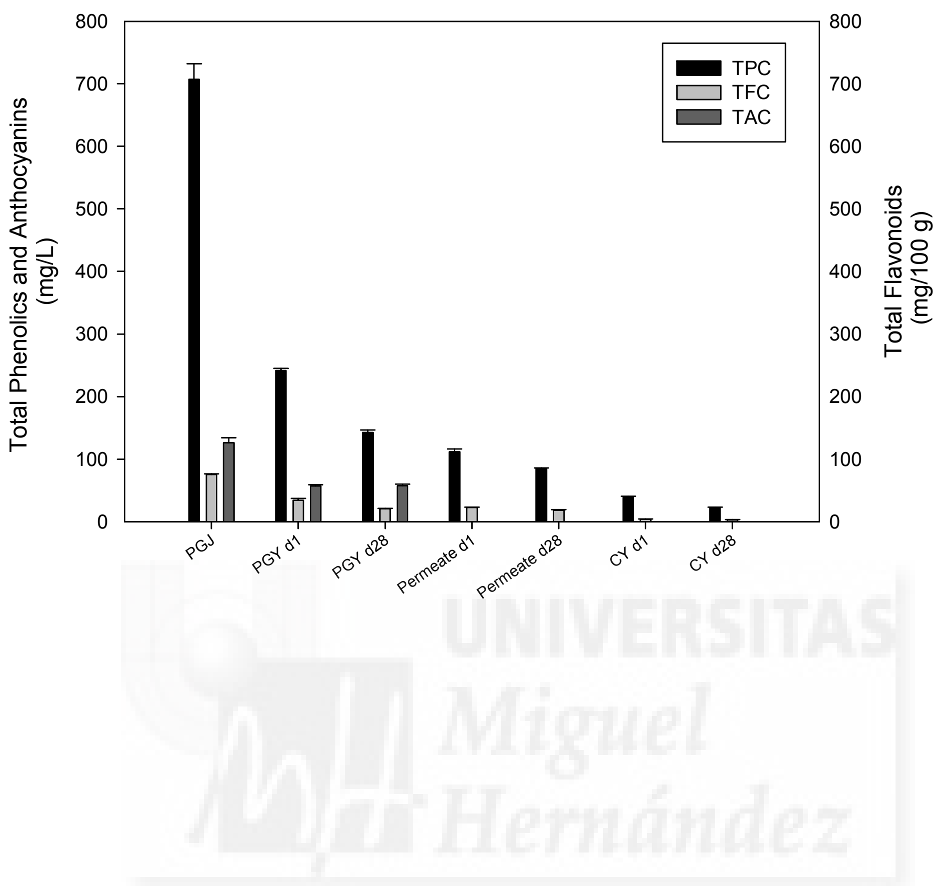


Figure 3



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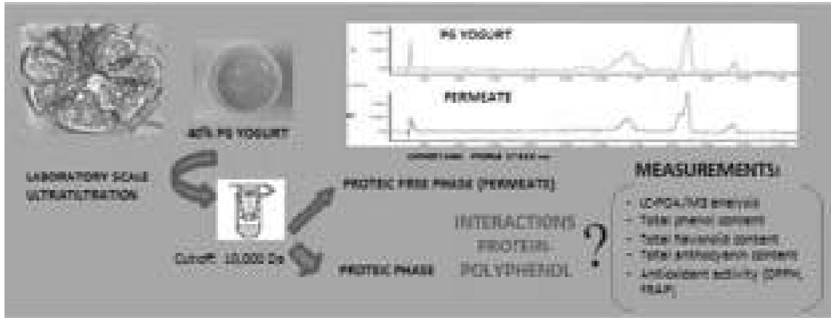
PG YOGURT
PERMEATE
LABORATORY SCALE ULTRAFILTRATION
PROTEIC FREE PHASE (PERMEATE)
Cutoff: 10,000 Da
PROTEIC PHASE
INTERACTIONS PROTEIN-POLYPHENOL
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MEASUREMENTS:

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PUBLICACIÓN 6

Food ingredients as anti-obesity agents: A review

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Food Ingredients as Anti-Obesity Agents: A Review

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Overweight and obesity have a major impact on global health; their prevalence has rapidly increased in all industrialized countries in the past few decades and diabetes and hypertension are their direct consequences. Pharmacotherapy provides reinforcement for obesity treatment, but should be an adjunctive support to diet, exercise, and lifestyle modification. At present, only orlistat and sibutramine have been approved by the US Food and Drug Administration for long-term use, but sibutramine was withdrawn for sale by the European Medicines Agency. The development of functional foods for the prevention and/or treatment of obesity suppose an opportunity for the food market and involve the knowledge of the mechanisms of appetite and energy expenditure as well as the metabolic sensation of satiety. Strategies for weight control management affect gut hormones as potential targets for the appetite metabolic regulation, stimulation of energy expenditure (thermogenesis), and modifications in the metabolic activity of the gut microbiota. Functional foods for obesity may also include bioactive fatty acids, phenolic compounds, soybean, plant sterols, dietary calcium, and dietary fiber. This review intends to offer an overview of the present situation of the anti-obesity agents currently used in dietary therapy as well as some functional food ingredients with potentially anti-obesity effects.

Keywords Obesity, pharmacology, fatty acids, phenolics, calcium, fiber

INTRODUCTION

Obesity is one of the major health problems worldwide, and it is a risk factor for several chronic disorders. Table 1 shows the prevalence of obesity in several world regions. The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk to health. The body mass index (BMI) is the most commonly used measure of obesity and is calculated from a person's height and weight (BMI = kg m^{-2}). For adults, overweight is currently defined as a BMI between 25 and 29.9 kg m^{-2} and obesity as a BMI equal to or greater than 30 kg m^{-2} (normal BMI = 20–25 kg m^{-2}) (WHO, 1998). Nevertheless, BMI independently does not provide information on body fat or body distribution. The risks associated with obesity are hypertension, dyslipidemia, type 2 diabetes, cardiovascular diseases (CVD), and obstructive sleep apnea (Sherwood and Story, 2003). Bacher et al. (2009) reviewed the main contributors to outcome, and visceral obesity is an individual risk factor for myocardial infarction and abdominal fat accumulation alone increases cardiovascular risk so waist circumference, a surrogate marker for abdominal fat, may predict the development of those risks.

A number of new epidemiological studies carried out in the United States demonstrate that the incidence of obesity is beginning to plateau. However, the prevalence of overweight among children and the rate of severe obesity in adults continue to grow, suggesting that hereafter there will be an increasing load of obesity-related illnesses (Bessesen, 2008). One billion adults are overweight and more than 300 million are obese. At least 2.6 million people each year die as a result of being overweight or obese. Globally, over 42 million children under five years of age are overweight; childhood obesity is one of the most serious public health challenges of the 21st century (WHO, 2010). In Spain, prevalence rates in school-aged population doubled in the past 15 years. In adults, prevalence is higher among women, particularly older than 40 years, and among those in lower socioeconomic and educational environments (Quiles-Izquierdo et al., 2008). Obesity may contribute to as high as 18,000 deaths

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Table 1 Prevalence of overweight and obesity in WHO regions, 2004

	Prevalence measure[a]		
Region	Mean BMI[b] (kg m^{-2})	Overweight and obesity (BMI ≥ 25) (%)	OBESE (BMI ≥ 30) (%)
World	24.5	42	12
Africa	23.0	30	6
Europe	26.9	65	24
The Americas	27.9	70	33
Eastern Mediterranean	25.2	48	18
South-East Asia	22.1	22	2
Western Pacific	23.4	31	3

[a]For person aged ≥30 years.
[b]BMI = body mass index is defined as weight (kg) divided by height (m) squared. Adapted from WHO, 2009.

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a year in Spain, being responsible for 5.5% of overall mortality (Banegas et al., 2003).

Complex interactions among neuroanatomical, genetic, endocrinological, pathophysiological, nutritional, physical, psychological, and social-environmental factors are the reason of energy balance in humans (Isidro and Cordido, 2010). The best opportunity to address the epidemic of obesity is to understand the underlying mechanisms of appetite control (Vincent and le Roux, 2007), applying this knowledge to help develop functional ingredients that can promote more substantial weight loss seems to be a promising concept to combat obesity in future.

In this study, we critically review the present situation of the anti-obesity agents currently used in dietary therapy as well as some functional food ingredients with potentially anti-obesity effects.

PHARMACOTHERAPY TREATMENT FOR OBESITY

Originally, mainly drugs that have inserted the market for obesity treatment were developed to treat psychiatric diseases (Adan et al., 2008). Treatments were initially target on immediate and relatively rapid weight reduction based around centrally acting amphetamine derived compounds; as a result, addiction, myocardial infarction, and stroke were the main side effects (Bacher et al., 2009). In 1990s, concerns were raised about the safety of two of these widely used weight management drugs, namely fen-phen (mixture of fenfluramine and phentermine) because of their association with valvulopathy (Connolly et al., 1997). As a result, the manufacturer agreed to withdraw the treatment from the market.

Progress in the pharmaceutical industry have resulted from the recognition of the need for long-term drug treatment for weight loss and resulted in the development of the three major compounds: orlistat, sibutramine, and rimonabant (Bacher et al., 2009). At present, only two medications are approved for long-term use worldwide (by the US Food and Drug Administration), orlistat (Xenical®) and sibutramine (Meridia®, Reductil®). However, sibutramine's marketing authorizations were suspended by the European Medicines Agency (EMEA) in January 2010 due to concerns on heart problems (Hsu et al., 2010). Rimonabant (Acomplia®) was approved by the EMEA in April 2006 but officially withdrawn in October 2008 because of its psychiatric side effects (EMEA, 2008).

Orlistat is a reversible gastrointestinal lipase inhibitor which prevents absorption of dietary fat (approximately 30%) by inactivating its hydrolyzation, thus reducing the calorie intake (Li and Cheung, 2009). Orlistat has been studied in several randomized controlled trials. Padwal et al. (2003) has pooled results from double-blind randomized controlled long-term studies and showed that all 11 orlistat prospective trials identified showed greater reduction in body weight than placebo-treated patients. Patients treated with orlistat presented a 2.7 kg weight loss after 1-year duration. A meta-analysis estimated a reduction in body weight of 2.59 kg after 6 months and 2.89 kg after 12 months, and the most weight loss occurs within the first 6 months of treatment (Li et al., 2005). Side effects of orlistat are thus related to the blockade of triglyceride (TG) digestion in the intestine because of orlistat is not absorbed to any significant degree from the gastrointestinal tract (Bray and Greenway, 2007).

Sibutramine is a centrally-acting serotonin and noradrenaline reuptake inhibitor that decreases food intake by enhancing satiety (Heal et al., 1998; Halford et al., 2010). Sibutramine also stimulates thermogenesis; although this secondary action plays a small part in weight loss (Lean, 2001). In a meta-analysis carried out by Arterburn et al. (2004) obese and overweight patients lost 4.45 kg on average after 12 months sibutramine treatment. In the STORM (Sibutramine Trial of Obesity Reduction and Maintence) study, obese patients who were taking 10 mg sibutramine with low calorie diet achieved 10% weight loss in the 6-month weight-loss phase but the group that did not continue with the sibutramine treatment appeared to regain weight in the following 18 months (James et al., 2000). Treatment with sibutramine in some patients, as a consequence of its peripheral mode of action, may result in small increases in blood pressure and heart rate (Padwal and Majumdar, 2007). Czernichow et al. (2010) conducted a meta-analysis in overweight adolescents of the efficacy of orlistat and sibutramine. They reviewed a total of eight randomized trials (three orlistat and five sibutramine). Overall, these drugs were associated with 5 cm reduction in waist circumference and 5.25 kg weight loss after at least six months of therapy compared with placebo, and no relevant cardiovascular side effects were detected.

Rimonabant, the first cannabinoid type 1 (CB_1) receptor blocker, is a potent CB_1-selective ligand, with 1000-fold greater affinity for the CB_1 receptor than the CB_2 receptor (Padwal and Majumdar, 2007). The endocannabinoid system plays an important role in the physiological regulation of food intake, energy balance, as well as lipid and glucose metabolism (Di Marzo et al., 2004). Treatment with rimonabant produces weight loss and decreases food intake in animals, and large-scale clinical trials showed its efficacy in treating obesity and dyslipidemia (Di Marzo, 2008). Compared with placebo, rimonabant

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significantly reduces weight by 4–5 kg on average, reduces waist circumference, and improves TG and HDL cholesterol profiles (Padwal and Majumdar, 2007). However, clinical data revealed serious side effects as depressive symptoms and anxiety (Van Gaal et al., 2005). Nevertheless, the knowledge of the physical and functional relationships between CB_1 receptors and interacting proteins could provide novel targets for drug discovery (Smith et al., 2010).

Ultimately, pharmacotherapy to tackle obesity is required as adjunctive support to diet, exercise, and lifestyle modification (Heal et al., 2009), and the choice of the obesity treatment is largely based on the preferences of each patient, clinical experience, and adverse effect profiles (Hsu et al., 2010). It should be taken into account that obesity drugs are usually expensive and can have severe side effects which are often not evident on initial release.

STRATEGIES FOR DEVELOPING ANTI-OBESITY FOODS

Most recent advances in food and nutrition sciences have highlighted the possibility of modulating some specific physiological functions in the organism through food intake (Jiménez-Colmenero, 2007). The development of functional foods for weight control involves the knowledge of the body weight control system. Serrano and Sánchez-Gonzalez (2008) reported the main strategies for body weight control by incorporating functional ingredients: inhibition of food intake (by inhibiting orexigenic signals or enhancing anorexigenic signals), limiting the bioavailability of nutrients (by suppressing the digestive enzymes and/or interacting with them to physically prevent their absorption), stimulation of energy expenditure (EE) (thermogenesis), and modifying the composition of the gut microbiota.

The gastrointestinal tract is an important source of metabolic signals, so plays a key role in sensing and signaling food intake to the brain (Neary and Batterham, 2009). Gut hormones are strategic mediators of this information, so a number of peptides synthesized in the gastrointestinal tract have been investigated as potential targets for the appetite metabolic regulation. The possibility of developing active ingredients that stimulate and/or inhibit the secretion of these peptides or acting at the receptor level made them an attractive strategy. So far, relatively few gut hormones have been completely investigated for their effects on fine-tune appetite and EE. Those found to affect food intake are peptide YY3-36 (PYY3-36), pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1), oxyntomodulin (OXM), gherlin, amylin, and cholecystokinin (CCK) (Moran and Dailey, 2009; Neary and Batterham, 2009). Gherlin stimulates appetite and food intake, in contrast all other known gut hormones have anorectic effects: they promote “satiation” (cause meal cessation) and/or promote “satiety” (delay the initiation of the next meal) (Moran at al., 2005).

Some food ingredients may promote a stimulatory effect on human energy EE and enhance satiety (Belza et al., 2009). Adaptive thermogenesis is a set of mechanisms that allow energy dissipation from foodstuff in a regulated manner as heat instead of accumulating as fat (Picó et al., 2006). The mechanism may be an enhancement of sympathetic nervous system (SNS) activity through increasing the noradrenaline level, which enhanced satiety and increased EE, caused suppression of hunger, mediated in part by increased fat oxidation (Belza et al., 2007).

The human body is home to a large number of distinct microbial communities, with the densest population in the distal gut (the gut microbiota). Evidence obtained in experimental models and human subjects are in accordance of the fact that changing the gut microbiota (with prebiotics and/or probiotics) may participate in the control of the development of metabolic diseases associated with obesity (Cani and Delzenne, 2009). Evidences propose that the metabolic activities of the gut microbiota facilitate the extraction of calories from indigested dietary substances, afterward stored in host adipose tissue for later use (DiBaise et al., 2008). Cani et al. (2008) suggested different mechanisms to explain the metabolic shift towards energy storage: (1) gut microbiota can increase the capacity to harvest energy from the diet and (2) gut microbiota can modulate plasma lipopolysaccharides levels which activate the inflammatory tone and the onset of obesity and type 2 diabetes. Nevertheless, progress in understanding the mechanisms by which the gut microbiota interact with the host will provide new basis for putative pharmacological or dietary intervention. Also, interventions intended at improving health parameters through microbiota modifications with pre- and probiotics supplements have often been short-term. Hence, knowledge about the effects of microbiota changes on long-term health is now necessary (Mai and Draganov, 2009).

FUNCTIONAL INGREDIENTS FOR WEIGHT LOSS

Table 2 summarizes main bioactive food ingredients which will be subsequently reviewed. A definition for bioactive compound was adopted by consensus in the 23rd Hohenheim Consensus Meeting defining bioactive compounds as essential and nonessential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain and can be shown to have an effect on human health (Biesalski et al., 2009).

Bioactive Fatty Acids

Lipids are among the bioactive components that have received most attention especially (in quantitative and qualitative terms) for the development of healthier meat products (Jiménez-Colmenero, 2007). Moreover, it is well known that the quality of dietary lipids could be an important modulator in terms of

Table 2 Summary of food ingredients with potential anti-obesity effects

Compound	Mechanism or hypothetical mechanism of action	Proven in animals/humans?	References
PUFAs *n*-3	Inhibiting key enzymes for lipid synthesis, increasing thermogenesis and preventing lipogenesis.	↓ CVD ↓ Obesity ?	Willumsen et al., 1993; Froyland et al., 1997; Kim et al., 1999, 2003, 2006; Guo et al., 2005; Liu et al., 2005; Buckley and Howe, 2009; Melason et al., 2009.
MUFAs	Enhancing β-oxidation of lipolytic enzymes activities and lowering plasma leptin concentrations.	↓ CVD Obesity →mixed results.	Tsunoda et al., 1998; Shai et al., 2008; Vögler et al., 2008; Melanson et al., 2009; Liao et al., 2010.
	VA → similar to CLA. Both fatty acids may regulate intestinal or hepatic lipogenic pathways.	↓ CVD in animals ↓ Obesity in animals → ?	Wang et al., 2008, 2009, 2010; Jacome-Sosa et al., 2010.
CLA	Reducing lipoprotein lipase activity and increasing enzymes associates with β-oxidation of lipids.	↓ Obesity in animals ↓ Obesity in humans → mixed results.	Azain et al., 2000; Blankson et al., 2000; DeLany and West, 2000; Jahreis et al., 2000; Smedman and Vessy, 2001; Thom et al., 2001; Meadus et al., 2002; Larsen et al., 2003; Nagao and Yanagita, 2005; Silveira et al., 2007.
Phenolic compounds	Catechins → increasing thermogenesis in brown adipose tissue.	↓ Obesity in animals ↓ Obesity in humans → moderate and short-term effects.	Dullo et al., 2000; Murase et al., 2002; Kovacs et al., 2004; Zheng et al., 2004; Westerterp-Platenga et al., 2005; Picó et al., 2006; Ito et al., 2008; Huang et al., 2009.
	Saponins → inhibiting pancreatic lipase activity, so inhibiting the intestinal absorption of fat.	↓ Obesity in animals Obesity in humans → n. p.	Han et al., 2001, 2005; Okuda and Han, 2001; Kim et al., 2009.
	Anthocyanins → regulating the adipocyte function.	↓ Obesity in animals Obesity in humans → n. p.	Tsuda et al., 2003, 2005, 2008.
	LFO → regulating the key enzymes involved in fatty acid oxidation and synthesis in liver; regulating PPAR-γ agonistic activity.	↓ Obesity in animals Obesity in humans → ?	Nakagawa et al., 2004; Kamisoyama et al., 2008.
Soybean	Isoflavones → modulating selective estrogen receptor activity.	↓ CVD ↓ Obesity in animals	Bathena and Velasquez, 2002; Munro et al., 2003; Velasquez and Bathena, 2007; Jang et al., 2008; Cederroth and Nef, 2009.
	Peptides → activating AMPK and phosphorilating hypothalamic STAT3.	↓ Obesity in humans → ?	
Plant sterols	Interfering with intestinal fatty acid absorption.	↓ CVD ↓ Obesity in animals ↓ Obesity in humans → ?	Suzuki et al., 2007; Takeshita et al., 2007; Rideout et al., 2010.
Dietary calcium	Increasing fecal fat excretion; regulating adipocyte metabolism and TAG storage; regulating UCP2 expression.	↓ Obesity in animals ↓ Obesity in humans → ?	Zemel et al., 2000; Shi et al, 2001; Shi et al., 2002; Zemel, 2002, 2005; Sun and Zemel., 2004; Jacobsen et al., 2005; Christensen et al., 2009; Astrup et al., 2010; Derbyshire, 2010.
Dietary fiber	Acting as a physiologic obstacle to energy intake; promoting secretion of anorexigenic peptides.	↓ CVD ↓ Obesity → mixed results.	Pereira and Ludwig, 2001; Liese et al., 2005; Murakami et al., 2007; Anderson, 2008; Astrup et al., 2010.

Abbrev: PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; CVD, cardiovascular diseases; VA, trans-11 vaccenic acid; CLA, conjugated linoleic acid; LFO, licorice flavonoid oil; PPAR-γ, peroxisome proliferator-activated receptor-γ; AMPK, AMP-activated protein kinase; STAT3, signal transducers and activators of the transcription 3, TAG, triacylglycerol; UCP2, uncloping protein-2.
↓ = decrease; ? = possible; n. p. = not proven.

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the morbidity and mortality of lifestyle-related diseases (Nagao and Yanagita, 2005).

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) can be classified in *n*-3 and *n*-6 fatty acids. The predominant dietary *n*-6 fatty acid is arachidonic acid and typical *n*-3 fatty acids are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). According to dietary recommendations for the intake of specific fatty acids as a proportion of total diet energy, 6–10% should be from PUFAs (*n*-6, 5–8%; *n*-3, 1–2%) (WHO, 2003). Major sources of *n*-6 PUFAs are vegetable oils (safflower, sunflower, sesame, soybean, and corn oils) for 18:2*n*-6 α-linoleic acid and meats and dairy products for 20:4*n*-6 arachidonic acid; fish oil is the main

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source for the n-3 PUFAs, 20:5n-3 EPA and 22:6n-3 DHA, and seed oils (linseed, canola, flax, and other seed oils) for 18:3n-3 α-linolenic acid (ALA) (Shireman, 2003).

The enrichment of frequent and common consumed food products appears to be a good way to raise the n-3 PUFA content in the diet (Kolanowski et al., 1999). The most obvious way of increasing intakes of EPA and DHA seems to be increasing consumption of oil-rich fish and fish products. Although, this presents a number of barriers as unpleasant taste, concerns about the sustainability of global fish stocks, vegans and vegetarians' beliefs, among others (Lunn and Buttriss, 2008). Therefore, focuses on other outcomes of fortified foods are commonly investigated. Techniques like microencapsulation (Luff, 2007; Drusch and Mannino, 2009; Serfert et al., 2009), the genetic modification of oilseeds to produce the fatty acids found in fish oils (Napier and Sayanova, 2005; Graham et al., 2007;), and the incorporation of fish oil in the animal's diet (Baer et al., 2001; Moghadasian, 2008) have been tested.

The potential anti-obesity effects of PUFAs may be explained through their performance on the following aspects: the balance between energy intake and EE, lipid metabolism, the status of adipocytes, and neuroendocrine system (Li et al., 2008). They inhibit key enzymes responsible for lipid synthesis, such as fatty acid synthase and stearoyl-CoA desaturase-1 (Kim et al., 1999, 2003), enhance lipid oxidation and thermogenesis (Willumsen et al., 1993; Froyland et al., 1997; Guo et al., 2005), and prevent free fatty acids from entering adipocytes for lipogenesis (Liu et al., 2005; Kim et al., 2006). However, despite a large amount of investigations, the precise molecular mechanisms responsible for their anti-obesity effects remain largely unknown. Animal studies have shown that incorporating n-3 long chain PUFA (LCPUFA) into high-fat diets designed to induce obesity reduces body fat accumulation, and reduce body fat weight in adult animals that are already obese (Buckley and Howe, 2009). Recently, Okada et al. (2011) have developed a capsule made with phospholipids (PLs) derived from scallop industry rich in LCPUFAs. Capsules contained *Undaria pinnatifida* (brown seaweed) lipids, which in turn included flucoxanthin, a well-demonstrated anti-obesity pigment (Maeda et al., 2007). The administration of the capsules to a mice model reduced body weight, adipose tissue mass, and promoted UCP1 expression showing a synergistic anti-obesity effect. In spite of promising results from animal studies that suggest that n-3 LCPUFA may reduce body fat, there are relatively little data available from well-controlled studies in humans. Melanson et al., (2009) reviewed studies from 1993 to 2009 regarding associations between intake of dietary fat and obesity, metabolic syndrome, and diabetes. Authors did not find a clearly association between the intakes of monounsaturated fatty acids (MUFAs) or PUFAs and body weight loss. On the other hand, there was enough number of scientific studies suggesting that total fat and saturated fat intake increase the risk of having components of the metabolic syndrome, and that higher intake of MUFAs or PUFAs have a beneficial effect in reducing this risk.

Vegetable oils (such as olive oil, canola oil, peanut oil, sunflower oil, and sesame oil), avocados, nuts, and seeds are the major dietary sources of MUFAs (Melanson et al., 2009). Olive oil has already shown a beneficial effect on coronary heart disease risk factors, attributed now not only because of a high MUFA level, mainly oleic acid (18:1n-9), also for a multiple minor components with biological properties (Covas et al., 2006). Several studies have been conducted among the association between MUFA consumption and body weight loss with mixed results. Recently, Liao et al. (2010) reported in a hamster obese-induced model that MUFAs decreased body fat deposition by lowering plasma leptin concentrations and enhancing β-oxidation of lipolytic enzymes activities, but it was highly dependent on the level of polyunsaturated-to-saturated fatty acid ratio. However, a high MUFA diet induced obesity in a mice model (Tsunoda et al., 1998). Shai et al. (2008) showed that moderate obese adults fed with and restricted-calorie Mediterranean dietary plan (which included MUFA) achieved more weight loss than did a low fat diet. However, in all cases, it is interesting to carefully consider the study design (sample size, duration of the study, follow-up, among others).

The study carried out by Vögler et al. (2008) showed that slight changes in the chemical structure of a fatty acid can influence its impact on body weight. This study investigated the relationship between chemical structure and physiological effect of the closely related C18 fatty acids (stearic acid: saturated, elaidic: Δ9-*trans*-monounsaturated, oleic: Δ9-*cis*-monounsaturated, linoleic: Δ9-cis-Δ12-cis-polyunsaturated and 2-hydroxy-Δ9-*cis*-octadecenoic acid (2-OHOA): synthetic Δ9-cis-monounsaturated) and the ability to reduce body weight in rats. It showed that only *cis*-configured monounsaturated fatty acids (oleic and 2-OHOA) had the ability to reduce body weight, adipose tissue mass and food intake, but only 2-OHOA imply the expression of uncoupling protein (UCP1) in white adipose tissue, suggesting the possibility of a process of transdifferentiation of white adipose tissue in a tissue with similarities to the brown adipose tissue accompanied with increased EE. Moreover, all those reductions were significantly enhanced more than four times with the introduction of the hydroxyl group in the alpha position of oleic acid. Furthermore, in regard to the reduction of body weight, 2-OHOA began to be effective at lower doses than oleic acid (Vögler et al., 2008).

Trans-11 vaccenic acid (VA), *trans*-11 18:1n-9, with the exception of a *trans* double bond at the C11 position, has a similar structure to that of oleic acid (*cis*-9, 18:1n-9). VA is the precursor of the endogenous synthesis of the *cis*9, *trans*11-conjugated linoleic acid (CLA) isomer in rats and humans, and is the predominant isomer of the total *trans* fatty acids found in ruminant derived fats such as dairy and meat products (Jacome-Sosa et al., 2010). Wang et al. (2008) short-term fed selected rats (JCR:LA-*cp*) which spontaneously develop symptoms associated with metabolic syndrome providing a diet with 1.5% (wt:wt) of VA. Dietary VA administration significantly decreased concentrations of fasting plasma triacylglycerols (TAGs). Later, Wang et al. (2009) in a similar long-term study fed same rat models

with a diet containing 1% (wt:wt) of VA. VA-fed rats showed signs of improving features of the abnormal retention of lipids within the liver. Also, results showed a substantial improvement in plasma lipid profile and abnormal non-fasting (postprandial) TAG and chylomicron concentrations. Furthermore, in the development of a number of chronic diseases, including obesity, an impaired metabolism of lipoproteins following a meal (e.g., chylomicrons) has been demonstrated (Wang et al., 2010). In the study of Jacome-Sosa et al. (2010) same type of rats fed with a combination of VA plus conjugated linoleic acid (CLA) showed a more reduced body weight than rats fed only with CLA.

Conjugated Fatty Acids

Conjugated fatty acids (CFAs) are a mixture of positional and geometric isomers of PUFAs with conjugated double bonds.

Conjugated linoleic acid (CLA), the CFA form of linoleic acid, has been naturally detected in milk fat, cheese, and ruminant meat (Sehat et al., 1998). In animal studies, CLA reduced body fat and increased lean body mass in growing animals, for a number of species, including mice, rats, and pigs (Azain et al., 2000; DeLany and West, 2000; Meadus et al., 2002). These effects were attributed to a reduction in lipoprotein lipase activity and an increase in the enzyme carnitine palmitoyl transferase (associated with β-oxidation of lipids) (Jahreis et al., 2000). Growing evidences indicate that individual isomers of CLA have specific physiological functions, for instance, the 10*trans*,12*cis*-CLA isomer has anticarcinogenic, antiobese, and antidiabetic effects, whereas the 9*cis*,11*trans*-CLA isomer exerts an anticancer effect (Nagao and Yanagita, 2005). Studies in humans have shown that supplementation with mixed isomers of CLA reduces body weight (Blankson et al., 2000; Thom et al., 2001) and percentage of body fat (Smedman and Vessy, 2001) but it is still unknown if all effects on humans are positive. The review carried out by Larsen et al., (2003) evaluated the efficacy and safety of CLA dietary supplements. Authors pooled data from 13 randomized placebo-controlled human intervention trials lasting more than four weeks; results showed little evidence to support a reduction of body weight or repartitioning of body fat by CLA from short-term (<six months) human studies. Also, it suggested that the 10*trans*,12*cis*-CLA isomer may actually adversely affect human health causing liver hypertrophy and insulin resistance (Larsen et al., 2003). Another review supports that although evidence regarding effectiveness of *trans*-10,*cis*-12-CLA in animals to reduce adiposity and increase lean mass is largely proved, the efficacy of either *trans*-10,*cis*-12-CLA or 9*cis*,11*trans*-CLA in humans is not concluding (Silveira et al., 2007). As far as human consumption is concerned, a definite conclusion for CLA safety has not been reached yet.

Tsuzuki et al. (2005, 2006) studied the physiological functions of CFAs other than CLA. They designed conjugated EPA (CEPA) and conjugated DHA (CDHA) by alkaline isomerization of EPA and DHA with the hypothesis that a combination of conjugated double bonds and an *n*-3 highly unsaturated structure might have stronger anti-obesity effects. Results from short-term (four weeks) animal studies showed that administration of CEPA or CDHA suppressed lipid accumulation in liver and adipose tissue more than linoleic acid, CLA, EPA or DHA, as well as other healthy benefits.

Phenolic Compounds

Phenolic compounds are one of the most diverse groups of secondary metabolites in edible plants with many potent biological properties. So far they have not been considered necessary from the nutritional point of view (Puupponen-Pimiä et al., 2002). Polyphenols, present in large quantities in tea, coffee, red grapes, kidney beans, prunes, and red wine, are well-known displaying anticarcinogenic, antioxidant, antibacterial, and antiviral action (Rincón-León, 2003). Catechins are the predominant and most significant of all tea polyphenols, and the four major green tea catechins are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Owuor, 2003), those polyphenols are converted into thearubigins and theaflavins in black tea (Wolfram et al., 2006). Green tea catechins have been extensively investigated for their health benefits, while black tea extracts have not been studied until recently. In a recent study, Huang et al., (2009) found stronger effects achieved by a combination of different compounds; the synergistic effect of orange peel extract (OPE) with black tea extract (BTE) and caffeine (CF) was proved to render more effective anti-obesity actions in mice studied for a 10-week period than individual compounds. Interestingly, if compared individually, the order of anti-obesity activity was BTE > CF > OPE > EGCG. Green tea extracts promote thermogenesis in brown adipose tissue due to the interaction between catechins and caffeine with noradrenaline released by the SNS (Dulloo et al., 2000). Overall, the action of caffeine and catechins prolong the stimulatory effects of noradrenaline on the lipid and energetic metabolism (Picó et al., 2006). Studies from in vitro brown fat cell experiments show catechins and caffeine to be synergistic in stimulating thermogenesis (Dulloo et al., 2000). A meta-analysis evaluated the anti-obesity effects of green tea supplementation based on 11 selected long-term studies; authors concluded that catechins produced a little positive effect on body weight loss and its maintenance; also, caffeine intake and ethnicity showed to be moderators influencing the effects of catechins (Hursel et al., 2009). Studies in animal models have proved the anti-obesity effects of tea catechins in obese and non-obese subjects (Murase et al., 2002; Zheng et al., 2004; Ito et al., 2008) but human clinical trials for weight loss are much less encouraging. Kovacs et al. (2004) proved that green tea extract was no better than placebo in maintaining a 7.5% weight loss over 13 weeks; and those were confirmed in a similar study carried out by Westerterp-Plantenga et al. (2005).

Saponins, also known as triterpenoids, are classified as polyphenols, and are abundant in soybeans more than in any other legume (Rincón-León, 2003) and platycodins are a group of saponin glycosides from *Platycodon grandiflorum*. A number of studies have proved that crude saponins inhibited pancreatic lipase activity in vitro and in mice model (Han et al., 2001;

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Okuda and Han, 2001; Han et al., 2005), which means that saponins inhibited the intestinal absorption of dietary fat. In addition, the supplementation with SK1 (an edible saponin-rich compound from *Platycodi radix*) to obese mice reduced body weight and fat accumulation and increased fecal lipid excretion (Kim et al., 2009). Zhao et al. (2008) pointed out that the hypocholesterolemic effects of platycodins are not caused by reduced cholesterol absorption or synthesis, but they could play a role enhancing cholesterol excretion.

Ultimately, much attention has been focused on plant flavonoids that might be beneficial in reducing obesity (Kamisoyama et al., 2008). Wu et al. (2010) showed that a flavonoid-enriched extract, which had rutin and gallic acid as main components, targeted lipid-regulated enzymes and may be effective in preventing lipid accumulation in obese mice.

Anthocyanins are the major group of water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, beans, fruits, vegetables, and red wine; as a result, we ingest significant amounts of anthocyanins from plant-based daily diets (Tsuda, 2008). Tsuda et al. (2003) demonstrated that dietary anthocyanins suppressed the development of obesity in induced obese mice, and later proved that had a therapeutic advantage for the regulation of the adipocyte function which is responsible for the adipocytochine expression and insulin sensitivity (Tsuda et al., 2005).

Several studies have focused on licorice, the root of *Glycyrrhiza* species, and specifically on licorice flavonoid oil (LFO) which contained hydrophobic flavonoids from *Glycyrrhiza glabra* L. Nakagawa et al. (2004) demonstrated that administration of 2% LFO was effective in preventing obesity and diabetes in an obese diabetic animal model and observed in in vitro model through moderate peroxisome proliferator-activated receptor-γ (PPAR-γ) activity. Kamisoyama et al., (2008) reported that LFO decreased the weight of the abdominal adipose tissue and the levels of hepatic and plasma TAG by regulation of rate-limiting enzymes involved in fatty acid synthesis and oxidation in the liver.

Soybean

Isoflavones (hormone-like diphenolic compounds) are a large and very distinctive subclass of the flavonoids. They are more limited in plant kingdom than other flavonoids, as they are found almost only in legumes, especially in soybean (Puupponen-Pimiä et al., 2002). Several reports (humans and rodents) sustain the hypothesis that soy proteins or soy-derived phytoestrogens may be beneficial for the prevention of obesity and diabetes (Bhathena and Velasquez, 2002; Velasquez and Bhathena, 2007). But in contrast to rodent studies which primarily focus on the evaluation of soy-derived compounds on weight and fat loss, reports in human are oriented towards serum lipid analysis because of the importance of atherosclerosis and the risk in CVD (Cederroth and Nef, 2009). The review performed by Cederroth and Nef (2009) concluded that although in animal studies soy and phytoestrogens are effective at reducing adipose tissue and improving glucose uptake, data from human trials do not offer clear support. Additionally, a meta-analysis found that the isoflavones typically found in soy products, despite their possible oestrogenic effects, are safe for consumption (Munro et al., 2003). In soybean, isoflavones are tightly associated with proteins, and numerous studies have included the investigation of their anti-obesity effects separately to elucidate as to which is the bioactive compound that imparts this healthy benefit. The study of Jang et al. (2008) reported that an isoflavone-free peptide mixture from black soybean significantly decreased food intake in rats which was attributed to direct AMP-activated protein kinase (AMPK) activation and hypothalamic signal transducers and activators of the transcription 3 (STAT3) phosphorylation. From the mentioned review of Cederroth and Nef (2009), a considerable amount of evidences suggest that there are conflicting results about the effect of soy and phytoestrogens on human metabolism mainly due to not standardized experimental designs; but a number of studies reported beneficial effects on metabolic parameters. For instance, Dalais et al. (2003) treated postmenopausal women during three months resulting in a lower LDL and TG level. In addition, Wu et al. (2006) also showed that dietary supplementation with isoflavones during six months reduced fat mass in postmenopausal Japanese women.

Plant Sterols

Phytosterols, also called plant sterols, are extensively distributed in the plant kingdom, and β-sitosterol, campesterol, and stigmasterol are found in high concentrations in seeds such as corn, soybeans, sesame and rapeseed, and their oils (Suzuki at al., 2007). These plant stanols and sterol esters are able to block cholesterol absorption in the body, maybe decreasing the solubility of cholesterol and preventing it from binding to the bile (Heinemann et al., 1991; Ikeda and Sugano, 1998). Also, several human studies have proved that the cholesterol-lowering effects of plant sterol esters may differ significantly according to the food matrix (Clifton et al., 2004; Hansel et al., 2007). Although plant sterols are not commonly recognized as effective TG-lowering agents, evaluation of previously published clinical plant sterols reveals a variable, yet often-overlooked response of plasma TG concentrations (Rideout et al., 2010). Recently, Rideout et al., (2010) showed significantly reductions in hepatic and plasma TG concentrations followed by plant sterols consumption in a mice model even with increased hepatic lipogenic gene expression and de novo lipogenesis. The authors also suggest that the observed reduced body weight gain might be associated to the reduced fat absorption in response to plant sterols consumption. Suzuki et al., (2007) developed a phytostenone mixture which included campestenone (a 3-oxo derivative of campesterol) by the fermentation of phystosterol. Authors demonstrated that the administration of this fermentation product of plant sterols to mice reduced body weight dose-dependently, lowered the concentrations of serum and liver TG and total cholesterol, in addition to increase the lipid excretion in the feces. Takeshita et al., (2007) provided a mayonnaise rich

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in diacylglycerol oil containing phytosterols to Japanese men resulting in a decrease in abdominal, visceral and subcutaneous fat areas without adverse events including serum fat-soluble vitamins levels, which was previously reported by Saito et al. (2006).

Dietary Calcium

Dietary calcium (Ca^{2+}) seems to have a key role in the regulation of energy metabolism, via at least three different mechanisms, and data from several studies support an anti-obesity effect of dietary calcium (Zemel et al., 2000), although controversial results have been published about. Several authors support that a high intake of Ca^{2+} increases fecal fat and EE (Jacobsen et al., 2005; Christensen et al., 2009) and the increased excretion of fecal fat is most probably due to the formation of insoluble calcium-fatty acid soaps and/or binding of bile acids (Astrup et al., 2010). Other mechanism of action included in other studies (Zemel et al., 2000; Shi et al., 2001; Zemel, 2005) demonstrated a key role for intracellular Ca^{2+} in the regulation of adipocyte metabolism and TAG storage. Briefly, theoretically dietary Ca^{2+} modulates circulating calcitriol (1,25-dihydroxyvitamin D) levels that in turn regulates intracellular calcium which affects fat metabolism in human adipocytes (Zemel, 2005). Suppression of calcitriol with high-calcium diets decreased adipocyte intracellular Ca^{2+}, decreased fatty acid synthase, increased lipolytic activity and decreased adiposity (Zemel, 2002). A third mechanism to explain the anti-obesity effect of calcium is associated with the decreased calcitriol followed by calcium intake. This decreased calcitriol promoted UCP2 expression, which, in turn, increased apoptosis of adipocytes (Shi et al., 2002; Sun and Zemel, 2004). The grade of these effects notably depends on the source of dietary calcium; the effects are markedly higher when the source is a dairy product than when it comes from supplements of calcium carbonate (Zemel et al., 2000) and a part of this additional anti-obesity bioactivity is likely due to additional bioactive compounds such as whey proteins (Zemel, 2003), CLA (Belury et al., 2003) and branched chain amino acids (Layman, 2003). This theory is sustained by cellular mechanistic studies, animal studies, human epidemiological studies and clinical trials (Zemel, 2005). However, in a meta-analysis on calcium intake and body weight loss has not been shown that calcium intake is linked to greater weight loss (Barr, 2003). Additionally, a recent review by Derbyshire (2010) discusses the relationship between calcium intake and reduced fat mass. Of the 21 studies identified, 12 studies have found a positive association between Ca^{2+} intake and body fat composition; however nine studies reported no association. Therefore, more studies are needed to consider the effects of dietary Ca^{2+} on body weight loss.

Dietary Fiber

During the last years dietary fiber (DF) has gained an additional importance related to its use as functional ingredient. DF includes non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, non-digestible carbohydrates that have positive physiologic effects in humans (Slavin, 2005). Viscosity and fermentability are the two physicochemical properties that have been recognized as producing beneficial physiological effects (Astrup et al., 2010). DF acts as protective agent against CVD, diverticulosis, constipation, irritable colon, colon cancer and diabetes. These data were recently reported and summarized (Viuda-Martos et al., 2010a). A theoretical reason for the anti-obesity action of fiber proposed by Heaton (1973) is that fiber acts as a physiologic obstacle to energy intake by at least three mechanisms: (1) fiber displaces available calories and nutrients from the diet; (2) fiber increases chewing resulting in an expansion of the stomach and increased satiety; and (3) fiber decreases the absorption efficiency of the small intestine. On the other hand, the post-prandial response of appetite regulating hormones by different DF sources is less well studied. However, the existing studies indicate an anorexigenic effect of DF (Astrup et al., 2010), particularly fermentable DFs are more effective at promoting secretion of GLP-1, one of the main anorexigenic peptides, than non-fermentable fibers (Reimer et al., 2010). From epidemiological studies, it is well known that DF intake, especially intake of whole grains or cereal fiber, protect against development of obesity (Anderson, 2008). Regardless of some limitations, most cross-sectional studies are fairly consistent in demonstrating an inverse association between BMI and DF (Liese et al., 2005; Murakami et al., 2007), while some studies do not find such association (Stevens et al., 2002; Thane et al., 2007). Of the review conducted by Pereira and Ludwig (2001), most experimental human studies (17) showed a beneficial effect of DF on energy intake. Seven studies reported mixed effects, whereas three studies reported no effects of DF on satiety; also, authors found that fiber affects secretion of gut hormones. Astrup et al. (2010) examined the studies published over the last three decades evaluating the effects of an increased DF intake on body weight and composition. Twelve studies investigated the effects of DF-rich foods; only one study reported a tendency toward a change in body weight with oat-bran enriched products compared to control (He et al., 2004). Regarding studies exploring DF supplements, approximately two-thirds of the studies reported higher weight loss with DF supplement compared with placebo (although most studies used a hypocaloric diet), but no dose-response relationship was apparent across these studies. Finally, those observations led to the development of a potential market for fiber-rich products and ingredients for the dietetic and chemical industries (Rodríguez et al., 2006).

INFLUENCE OF FOOD PROCESSING ON THE STABILITY OF THE BIOACTIVE COMPOUNDS

To this point, we have focused on the bioactive ingredient itself, but it is important to keep in mind that food processing or technological treatments could influence the availability of

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the bioactive ingredient, since bioactive food components may suffer inactivation or degradation.

Ruiz-Rodriguez et al. (2008) reviewed the effect of domestic processing on the stability of several bioactive ingredients. PUFAs are not water-soluble compounds, so cooking methods involving water are less aggressive than those using oils as cooking medium. Baking or grilling demand even higher temperatures but losses seem to be not extremely high; these cooking methods provoke dehydration and a Maillard coating which might protect inside compounds. Phytosterols are also lipidic compounds and probably they behave as PUFAs, but reports are insufficient to draw conclusions. More polar compounds such as phenolics lower their content by water cooking processes, which is explained first by leaching and later by their thermal lability. Therefore, processes that involve high amounts of water, such as boiling, represent higher losses; other methods such as steaming, with almost no osmotic processes, are more recommended to retain a high level of these compounds inside the enriched food. DFs seem to be rather stable to cooking procedures including baking of dough although they are complex molecules affected by hot water.

Industrial treatments include many of the processes previously described and also others like toasting, kilning, drying processes, canning, pasteurization, and ultra high pressure; also, it is important to note that these processes can be controlled much better on industrial scale than on household level. Processing can have an effect on the solubility of the fiber by reducing its molecular weight, enzymatically or mechanically, for example, during extrusion by applying different shear forces. The consequences of food processing on the fate of the bioactive ingredient may differ largely according to many factors such as their concentration, chemical structure, oxidation state, possible interactions with other food components, and type of processing applied; in this sense, food processing may be responsible for a decrease, increase, or minor changes in content, and functionality of the bioactive component (van Boekel et al., 2010). The alkalinization process decreases the content of catechins in cocoa (Miller et al., 2008). Heating increases the amount of flavonoids in different vegetables (Miglio et al., 2008; Wachtel-Galor et al., 2008) and high-pressure technologies avoid degradation of anthocyanins in fruit juices (Klopotek et al., 2005). The study of Jackson et al. (2002) showed a decline in the content of isoflavones from raw soybean to soy beverage (losses of 46%) and tofu (losses of 64%) manufacture; these losses resulted in a considerable amount of isoflavones being lost in the resultant by-products. Similarly, Han et al., (2007) demonstrated that the

Table 3 Some examples of food products fortified with potential anti-obesity ingredients

Product category	Sub-type	Bioactive ingredient	References
Meat products[1]	Pork sausages	Green tea powder	Choi et al., 2003
	Dry fermented turkey sausage	Green tea extract	Bozkurt, 2006
	Raw sausage	SPI	Porcella et al., 2001
	Bologna sausage	Flavonoids	Viuda-Martos et al., 2009, 2010b
	Dry-cured sausage	Orange fiber	Fernández-López et al., 2008
Fish	Tuna 'pate'	Orange fiber	Sánchez-Zapata et al., 2009
	Minced fish product	Fiber	Cardoso et al., 2010
	Fish (heat-induced) gel	Inner pea fiber	Cardoso et al., 2007
Bread and bakery products	Bread	PUFAs	Yep et al., 2002
	Bread, biscuits, cake	PUFAs	Lovegrove et al., 1997
	Bars	PUFAs	Horn et al., 2009; Nielsen and Jacobsen, 2009
	Bread, cereal	PS	Clifton et al., 2004
Milk and dairy products	Milk	PUFAs	Let et al., 2005, 2007b
	Milk shake, milk shake powder	PUFAs	Lovegrove et al., 1997
	Drinking yoghurt	PUFAs	Nielsen et al., 2009
	Milk, yoghurt, fermented milk (low fat)	PS	Clifton et al., 2004; Hansel et al., 2007
	Yoghurt	Isoflavones	Rossi et al., 2008
	Yoghurt	Green and black tea	Jiménez et al., 2008; Jaziri et al., 2009
	Fermented milk	Citrus fibers	Sendra et al., 2008
Margarines and spreads	Filling for sandwich cookies	PUFAs	Borneo et al., 2007
	Spreadable fat	PUFAs	Kolanowsky et al., 2004
	Low-fat spread	PUFAs	Lovegrove et al., 1997
Mayonnaise and dressing	Mayonnaise	PUFAs	Lovegrove et al., 1997; Jacobsen et al., 1999, 2001;
	Salad dressing	PUFAs	Let et al., 2007a
	Mayonnaise	PS	Takeshita et al., 2007
Juices	Orange juice	PUFAs	Lovegrove et al., 1997
	Lemon juice	Anthocyanins and other phenolics	González-Molina et al., 2008

Abbrev: SPI, soy protein isolate; PUFAs, polyunsaturated fatty acids; PS, plant sterols.
[1]It has excluded meat and meat products fortified by dietary supplementation at animal production level.
[2]A raw sausage.

isoflavone content decreased from soybeans by steaming and boiling processes, increased during germination and fermentation, and decreased again in the soybean curd production.

Another point of view to take into consideration is the possibility of protecting the bioactive compounds by encapsulation. The encapsulation represents a promising solution for the bioactive compounds since many report low survival of bioactivity due to adverse effects of processing and storage in the vehicle food product and due to harmful circumstances during transport through the gastrointestinal tract (de Vos et al., 2010).

FINAL REMARKS

This review provides an extensive literature about the present situation of the pharmacological and dietary methods against the worldwide epidemic of obesity. The drugs presently available for the treatment of overweight patients are limited in efficacy and few in number, and although several drugs have promising anti-obesity effects, there are many aspects within this research field that need further investigation. Currently, what is clearly established is that exist a world in common foods to discover with great potential as functional ingredients that may produce anti-obesity effects. In this sense, food industry plays an important role in the development of healthier foods intended for weight control, and its success requires knowledge from different scientific disciplines, such as nutrition, biotechnology, consumer sciences, and multidisciplinary research projects in this area are thus required.

Strategies to fortify foods with bioactive compounds often require innovative approaches because of their sensitivity to a range of chemical and physical factors, and the food industry regulations with respect to ingredients, processing methods, and storage conditions are tight. Technologies for the delivery of active ingredients in foods include encapsulation, change in food formulation (re-formulation), and adaptation of the processing conditions; those are well-reported by Ubbink and Krüger (2006). Examples of fortified foods with bioactive ingredients are given in Table 3. However, the fact that the functional ingredient itself may be beneficial to human, it is not enough; so, further studies are needed to provide strong evidences for the human health benefits of the bioactive ingredients in the food matrix together.

Taking together with the increased scientific data, food scientists and industry have to spend more efforts in dissemination of information about healthier lifestyles that help to reverse the growing trend of obesity.

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REFERENCES

Adan, R. A. H., Vanderschuren, L. J. M. J. and la Fleur, S. E. (2008). Anti-obesity drugs and neural circuits of feeding. *Trends Pharmacol. Sci.* **29**(4):208–217.

Anderson, J. W. (2008). Dietary fiber and associated phytochemicals in prevention and reversal of diabetes. **In**: Nutraceuticals, Glycemic Health and Type 2 Diabetes, pp. 111–142. Pasupuleti, V. K. and Anderson, J. W., Eds., Blackwell Publishing Professional, Iowa.

Arterburn, D. E., Crane, P. K. and Veenstra, D. L. (2004). The efficacy and safety of sibutramine for weight loss: A systematic review. *Arch. Intern. Med.* **164**:994–1003.

Astrup, A., Kristensen, M., Gregersen, N. T., Belza, A., Lorenzen, J. K., Due, A. and Larsen, T. M. (2010). Can bioactive foods affect obesity?. *Ann. N. Y. Acad. Sci.* **1190**:25–41.

Azain, M. J., Hausman, D. B., Sisk, M. B., Flatt, W. P. and Jewel, D. E. (2000). Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. *J. Nutr.* **130**:1548–1554.

Bacher, H. P., Shepherd, G. M. and Legler, U. F. (2009). Pharmacological therapies of obesity: A review of current treatments options. *Biomol. Ther.* **17**(4):348–352.

Baer, R. J., Ryali, J., Schingoethe, D. J., Kasperson, K. M., Donovan, D. C., Hippen, A. R. and Franklin, S. T. (2001). Composition and properties of milk and butter from cows fed fish oil. *J. Dairy Sci.* **84**:345–353.

Banegas, J. R., López-García, E., Gutiérrez-Fisac, J. L., Guallar-Castillón, P. and Rodríguez-Artalejo, F. (2003). A simple estimate of mortality attributable to excess weight in the European Union. *Eur. J. Clin. Nutr.* **57**:201–208.

Barr, S. I. (2003). Increased dairy product or calcium intake: Is body weight or composition affected in humans? *J. Nutr.* **133**:245S–248S.

Belury, M. A., Mahon, A. and Banni, S. (2003). The conjugated linoleic acid (CLA) isomer, t10c12-CLA, is inversely associated with changes in body weight and serum leptin in subjects with type 2 diabetes mellitus. *J. Nutr.* **133**:257S–260S.

Belza, A., Frandsen, E. and Kondrup, J. (2007). Body fat loss achieved by stimulation of thermogenesis by a combination of bioactive food ingredients: A placebo-controlled, double-blind 8-week intervention in obese subjects. *Int. J. Obesity.* **31**:121–130.

Belza, A., Gille, M.-B., Schultz John, S. and Kondrup, J. (2009). The β-adrenergic antagonist propranolol partly abolishes thermogenic response to bioactive food ingredients. *Metabolism*. **58**:1137–1144.

Bessesen, D. H. (2008). Update on obesity. *J. Clin. Endocrinol. Metab.* **93**:2027–2034.

Bhathena, S. J. and Velasquez, M. T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr.* **76**:1191–1201.

Biesalski, H.-K., Dragsted, L. O., Elmadfa, I., Grossklaus, R., Müller, M., Schrenk, D., Walter, P. and Weber, P. (2009). Bioactive compounds: Definition and assessment of activity. *Nutrition*. **25**:1202–1205.

Blankson, H., Stakksetad, J. A., Fagertun, H., Thom, E., Wadstein, J. and Gudmundsen, O. (2000). Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J. Nutr.* **130**:2943–2948.

Borneo, R., Kocer, D., Ghai, G., Tepper, B. J. and Karwe, M. V. (2007). Stability and consumer acceptance of long-chain omega-3 fatty acids (eicosapentaenoic acid, 20:5, n-3 and docosahexaenoic acid, 22:6, n-3) in cream-filled sandwich cookies. *J. Food Sci.* **72**:S49–S54.

Bozkurt, H. (2006). Utilization of natural antioxidants: Green tea extract and *Thymbra spicata* oil in Turkish dry-fermented sausage. *Meat Sci.* **73**:442–450.

Bray, G. A. and Greenway, F. L. (2007). Pharmacological treatment of the overweight patient. *Pharmacol. Rev.* **59**:151–184.

Buckley, J. D. and Howe, P. R. C. (2009). Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids. *Obes. Rev.* **10**:648–659.

Cani, P. D. and Delzenne, N. M. (2009). The role of the gut microbiota in energy metabolism and metabolic disease. *Curr. Pharm. Design.* **15**(13):1546–1558.

Cani, P. D., Delzenne, N. M., Amar, J. and Burcelin, R. (2008). Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. *Pathol. Biol.* **56**(5):305–309.

Cardoso, C., Mendes, R. and Nunes, M. L. (2007). Dietary fibers' effect on the textural properties of fish heat-induced gels. *J. Aquat. Food Prod. T.* **16**:19–30.

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Cardoso, C., Mendes, R., Pedro, S., Vaz-Pires, P. and Nunes, M. L. (2010). Quality changes during storage of minced fish products containing dietary fiber and fortified with ω3 fatty acids. *Food Sci Technol Int*. **16**:31–42.

Cederroth, C. R. and Nef, S. (2009). Soy, phytoestrogens and metabolism: A review. *Mol. Cell. Endocrinol.* **304**:30–42.

Choi, S. H., Kwon, H. C., An, D. J., Park, J. R. and Oh, D. H. (2003). Nitrite contents and storage properties of sausage added with green tea powder. *Korean J. Food Sci. An.* **23**:299–308.

Christensen, R., Lorenzen, J. K., Svith, C. R., Bartels, E. M., Melanson, E. L., Saris, W. H., Tremblay, A. and Astrup, A. (2009). Effect of calcium from dairy and dietary supplements on faecal fat excretion: A meta-analysis of randomised controlled trials. *Obes. Rev.* **10**:475–486.

Clifton, P. M., Noakes, M., Sullivan, D., Erichsen, N., Ross, D., Annison, G., Fassoulakis, A., Cehun, M. and Nestel, P. (2004). Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. *Eur. J. Clin. Nutr.* **58**:503–509.

Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D. and Schaff, H. V. (1997). Valvular heart disease associated with fenfluramine-phentermine. *N. Engl. J. Med.* **337**:581–588.

Covas, M. I., Ruiz-Gutiérrez, V., De la Torre, R., Kafatos, A., Lamuela-Raventós, R. M., Osada, J., Owen, R. W. and Visioli, F. (2006). Minor components of olive oil: Evidence to date of health benefits in humans. *Nutr. Rev.* **64**:20–30.

Czernichow, S., Lee, C. M. Y., Barzi, F., Greenfield, J. R., Baur, L. A., Chalmers, J., Woodward, M. and Huxley, R. R. (2010). Efficacy of weight loss drugs on obesity and cardiovascular risk factors in obese adolescents: A meta-analysis of randomized controlled trials. *Obes. Rev.* **11**:150–158.

Dalais, F. S., Ebeling, P. R., Kotsopoulos, D., McGrath, B. P. and Teede, H. J. (2003). The effects of soy protein containing isoflavones on lipids and indices of bone resorption in postmenopausal women. *Clin. Endocrinol.* **58**:704–709.

de Vos, P., Faas, M. M., Spasojevic, M. and Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *Int. Dairy J.* **20**:292–302.

Derbyshire, E. (2010). Does adult calcium intake influence body composition? A review. *Nutr Food Sci*. **40**(5):494–503.

DeLany, J. P. and West, D. B. (2000). Changes in body composition with conjugated linoleic acid. *J. Am. Coll. Nutr.* **19**:487S–493S.

Di Marzo, V. (2008). CB_1 receptor antagonism: Biological basis for metabolic effects. *Drug Discov. Today.* **13**:1026–1041.

Di Marzo, V., Bifulco, M. and De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov*. **3**:771–784.

DiBaise, J. K., Zhang, H., Crowell, M. D., Krajmalnik-Brown, R., Decker, G. A. and Rittmann, B. E. (2008). Gut microbiota and its possible relationship with obesity. *Mayo Clin. Proc.* **83**(4):460–469.

Drusch, S. and Mannino, S. (2009). Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids. *Trends Food Sci. Tech.* **20**:237–244.

Dulloo, A. G., Seydoux, J., Girardier, L., Chantre, P. and Vandermander, J. (2000). Green tea and thermogenesis: Interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int. J. Obes.* **24**:252–258.

European Medicines Agency, EMEA. (2008). The European Medicines Agency recommends suspension of the marketing authorization of Acomplia. [press release] Oct 23, 2008. Available from: http://www.emea.europa.eu (Doc.Ref.: EMEA/CHMP/537777/2008).

Fernández-López, J., Sendra, E., Sayas-Barberá, M. E., Navarro, C., Pérez-Alvarez, J. A. (2008). Physicochemical and microbiological profiles of “Salchichón” (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Sci*. **80**:410–417.

Froyland, L., Madsen, L., Vaagenes, H., Totland, G. K., Auwerx, J., Kryui, H., Staels, B. and Berge, R. K. (1997). Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J. Lipid Res.* **38**:1851–1858.

González-Molina, Moreno, D. A. and García-Viguera, C. (2008). Aronia-enriched lemon juice: A new highly antioxidant beverage. *J. Agric. Food Chem*. **56**:11327–11333.

Graham, I. A., Larson, T. and Napier, J. A. (2007). Rational metabolic engineering of transgenic plants for biosynthesis of omega-3 polyunsaturates. *Curr. Opin. Biotech.* **18**:142–147.

Guo, W., Xie, W., Lei, T. and Hamilton, J. A. (2005). Eicosapentaenoic acid, but not oleic acid, stimulates beta-oxidation in adipocytes. *Lipids*. **40**:815–821.

Halford, J. C. G., Boyland, E. J., Cooper, S. J., Dovey, T. M., Huda, M. S. B., Dourish, C. T., Dawson, G. R. and Wilding, J. P. H. (2010). The effects of sibutramine on the microstructure of eating behavior and energy expenditure in obese women. *J. Psychopharmacol.* **24**:99–109.

Han, J.-S., Hong, H.-D., Kim, S.-R. (2007). Changes in isoflavone content and mass balance during soybean processing. *Food Sci. Biotechnol.* **16**(3):426–433.

Han, L.-K., Kimura, Y., Kawashima, M., Takaku, T., Hayashi, T., Zheng, Y.-N. and Okuda, H. (2001). Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. *Int. J. Obesity.* **25**(10):1459–1464.

Han, L.-K., Zheng, Y.-N., Yoshikawa, M., Okuda, H. and Kimura, Y. (2005). Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicas* rhizomes. *BMC Complem. Altern. M*. **5**:9.

Hansel, B., Nicolle, C., Lalanne, F., Tondu, F., Lassel, T., Donazzolo, Y., Ferrières, J., Krempf, M., Schlienger, J.-L., Verges, B., Chapman, M. J. and Bruckert, E. (2007). Effect of low-fat, fermented milk enriched with plant sterols on serum lipid profile and oxidative stress in moderate hypercholesterolemia. *Am. J. Clin. Nutr.* **86**:790–796.

He, J., Streiffer, R. H., Muntner, P., Krousel-Wood, M. A. and Whelton, P. K. (2004). Effect of dietary fiber intake on blood pressure: A randomized, double-blind, placebo controlled trial. *J. Hypertens.* **22**:73–80.

Heal, D. J., Aspley, S., Prow, M. R., Jackson, H. C., Martin, K. F. and Cheetham, S. C. (1998). Sibutramine: A novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from d-amphetamine and d-fenfluramine. *Int. J. Obesity.* **22**:S18–S28.

Heal, D. J., Gosden, J. and Smith, S. L. (2009). Regulatory challenges for new drugs to treat obesity and comorbid metabolic disorders. *Br. J. Clin. Pharmacol.* **68**(6):861–874.

Heaton, K. W. (1973). Food fibre as an obstacle to energy intake. *Lancet*. **2**:1418–1421.

Heinemann, T., Kullak-Ublick, G. A. and Pietruck, B. J. (1991). Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol. *Eur. J. Clin. Pharmacol.* **40**:59S–63S.

Horn, A. F., Nielsen, N. S. and Jacobsen, C. (2009). Addition of caffeic acid, ascorbyl palmitate or γ-tocopherol to fish oil enriched energy bars affects lipid oxidation differently. *Food Chem*. **112**:412–420.

Huang, Y.-W., Liu, Y., Dushenkov, S., Ho, C.-T. and Huang, M.-T. (2009). Anti-obesity effects of epigallocatechin-3-gallate, orange peel extract, black tea extract, caffeine and their combinations in a mouse model. *J. Funct. Foods.* **1**:304–310.

Hursel, R., Viechtbauer, W. and Westerterp-Plantenga, M. S. (2009). The effects of green tea on weight loss and weight maintenance: A meta-analysis. *Int. J. Obesity.* **33**:956–961.

Hsu, Y.-W., Chu, D.-C., Ku, P.-W., Liou, T.-H. and Chou, P. (2010). Pharmacotherapy for obesity: Past, present and future. *J. Exp. Clin. Med.* **2**(3):118–123.

Ikeda, I. and Sugano, M. (1998). Inhibition of cholesterol absorption by plant sterols for mass intervention. *Curr. Opin. Lipidol.* **9**:527–531.

Isidro, M. L. and Cordido, F. (2010). Approved and off-label uses of obesity medications, and potential new pharmacologic treatments options. *Pharmaceuticals*. **3**:125–145.

Ito, Y., Ichikawa, T., Morohoshi, Y., Saegusa, Y. and Ishihara, K. (2008). Effect of tea catechins on body fat accumulation in rats fed a normal diet. *Biomed. Res.* **29**(1):27–32.

Jacobsen, C., Hartvigsen, K., Lund, P., Meyer, A. S., Adler-Nissen, J., Holstborg, J. and Hølmer, G. (1999). Oxidation in fish oil enriched mayonnaise: 1. Assessment of propyl gallate as antioxidant by discriminant partial least squares regression analysis. *Eur. Food Res. Technol*. **210**:13–30.

Jacobsen, C., Hartvigsen, K., Thomsen, M. K., Hansen, L. F., Lund, P., Skibsted, L. H., Hølmer, G., Adler-Nissen, J. and Meyer, A. S. (2001). Lipid oxidation in

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fish oil enriched mayonnaise: Calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *J. Agric. Food Chem.* **49**:1009–1019.

Jacobsen, R., Lorenzen, J. K., Toubro, S., Krog-Mikkelsen, I. and Astrup, A. (2005). Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int. J. Obesity.* **29**: 292–301.

Jacome-Sosa, M. M., Lu, J., Wang, Y., Ruth, M. R., Wright, D. C., Reaney, M. J., Shen, J., Field, C. J., Vine, D. F. and Proctor, S. D. (2010). Increased hypolipidemic benefits of *cis*-9, *trans*-11 conjugated linoleico acid in combination with *trans*-11 vaccenic acid in a rodent model of the metabolic syndrome, the JCR: LA-*cp* rat. *Nutr. Metab*. **7**:60–70.

Jackson, C.-J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., Buzzell, D. and DeGrandis, S. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochem*. **37**:1117–1123.

Jahreis, G., Kraft, J., Tischendorf, F., Schöne, F. and von Loeffelholz, V. (2000). Conjugated linoleic acids: Physiological effects in animal and man with special regard to body composition. *Eur. J. Lipid Sci. Tech.* **102**:695–7103.

James, W. P. T., Astrup, A., Finer, N., Hilsted, J., Kopelman, P., Rössner, S., Saris, W. H. and Van Gaal, L. F.for the STORM Study Group. (2000). Effect of sibutramine on weight maintenance after weight loss: A randomised trial. *Lancet*. **356**:2119–2125.

Jang, E.-H., Moon, J.-S., Ko, J. H., Ahn, C.-W., Lee, H.-H., Shin, J.-K., Park, C.-S. and Kang, J.-H. (2008). Novel black soy peptides with antiobesity effects: Activation of leptin-like signaling and AMP-activated protein kinase. *Int. J. Obesity.* **32**:1161–1170.

Jaziri, I., Ben Slama, M., Mhadhbi, H., Urdaci, M. C. and Hamdi, M. (2009). Effect of green and black teas (*Camellia sinensis* L.) on the characteristic microflora of yogurt during fermentation and refrigerated storage. *Food Chem.* **112**(3):614–620.

Jiménez, A. M., Murcia, M. A., Parras, P. and Martínez-Tomé, M. (2008). On the importance of adequately choosing the ingredients of yoghurt and enriched milk for their antioxidant activity. *Int. J. Food Sci. Tech.* **43**:1464–1473.

Jiménez-Colmenero, F. (2007). Healthier lipid approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci. Tech.* **18**:567–578.

Kamisoyama, H., Honda, K., Tominaga, Y., Yokota, S. and Hasegawa, S. (2008). Investigation of the anti-obesity action of licorice flavonoid oil in diet-induced obese rats. *Biosci. Biotechnol. Biochem.* **72**(12):3225–3231.

Kim, H., Choi, S., Lee, H. J., Lee, J. H. and Choi, H. (2003). Suppression of fatty acid synthase by dietary polyunsaturated fatty acids is mediated by fat itself, not by peroxidative mechanism. *J. Biochem. Mol. Biol.* **36**:258–264.

Kim, H. K., Della-Fera, M., Lin, J. and Baile, C. A. (2006). Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. *J. Nutr.* **136**:3965–3696.

Kim, J.-Y., Moon, K.-D., Seo, K.-I., Park, K.-W., Choi, M.-S., Do, G.-M., Jeong, Y.-K., Cho, Y.-S. and Lee, M.-K. (2009). Supplementation of SK1 from platycodi radix ameliorates obesity and glucose intolerance in mice fed a high-fat diet. *J. Med. Food.* **12**(3):629–636.

Kim, H. J., Takahashi, M. and Ezaki, O. (1999). Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J. Biol. Chem.* **274**:25892–25898.

Klopotek, Y., Otto, K. and Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *J. Agric. Food Chem.* **53**:5640–5646.

Kolanowski, W., Swiderski, F. and Berger, S. (1999). Possibilities of fish oil application for food products enrichment with ω-3 PUFA. *Int. J. Food Sci. Nutr.* **50**(1):39–49.

Kolanowski, W., Swiderski, F., Jaworska, D. and Berger, S. (2004). Stability, sensory quality, texture properties and nutritional value of fish oil-enriched spreadable fat. *J. Sci. Food Agric.* **84**:2135–2141.

Kovacs, E. M., Lejeune, M. P., Nijs, I. and Westerterp-Plantenga, M. S. (2004). Effects of green tea on weight maintenance after body-weight loss. *Br. J. Nutr.* **91**:431–437.

Larsen, T. M., Toubro, S. and Astrup, A. (2003). Efficacy and safety of dietary supplements containing conjugated linoleic acid (CLA) for the treatment of obesity-evidence from animal and human studies. *J. Lipid Res.* **44**(12):2234–2241.

Layman, D. K. (2003). The role of leucine in weight loss diets and glucose homeostasis. *J. Nutr.* **133**:261S–267S.

Lean, M. E. (2001). How does sibutramine work? *Int. J. Obes. Relat. Metab. Disord.* **25**:S8–S11.

Let, M. B., Jacobsen, C., Frankel, E. N. and Meyer, A. S. (2007a). Ascorbyl palmitate, gamma-tocopherol, and EDTA affect lipid oxidation in fish oil enriched salad dressing differently. *J. Agric. Food Chem.* **55**:2369–2375.

Let, M. B., Jacobsen, C., Pham, K. A. and Meyer, A. S. (2005). Protection against oxidation of fish oil enriched milk emulsions through addition of rapeseed oil or antioxidants. *J. Agric. Food Chem.* **53**:5429–5437.

Let, M. B., Jacobsen, C., Sørensen, A. D. M. and Meyer, A. S. (2007b). Homogenization conditions affects the oxidative stability of fish oil enriched milk emulsions: Lipid oxidation. *J. Agric. Food Chem.* **55**:1773–1780.

Li, M. and Cheung, B. M. Y. (2009). Pharmacotherapy for obesity. *Br. J. Clin. Pharmacol.* **68**(6):804–810.

Li, J.-J., Huang, C. J. and Xie, D. (2008). Anti-obesity effects of conjugated linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. *Mol. Nutr. Food Res.* **52**:631–645.

Li., Z., Maglione, M., Tu, W., Mojica, W., Arterburn, D., Shugarman, L. R., Hilton, L., Suttorp, M., Solomon, V., Shekelle, P. G. and Morton, S. C. (2005). Meta-analysis: Pharmacologic treatment of obesity. *Ann. Intern. Med.* **142**:532–546.

Liao, F.-H., Liou, T.-H., Chiu, W.-C., Shieh, M.-J. and Chien, Y.-W. (2010). Differential effects of high MUFA with high or low P/S ratio (polyunsaturated to saturated fatty acids) on improving hepatic lipolytic enzymes and mediating PPARγ related with lipoprotein lipase and hormone-sensitive lipase of white adipose tissue in diet-induced obese hamster. *Int. J. Obesity.* **34**:1608–1617.

Liese, A. D., Schulz, M., Fang, F., Wolever, T. M. S., D'Agostino Jr., R. B., Sparks, K. C. and Mayer-Davis, E. J. (2005). Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the Insulin Resistance Atherosclerosis Study. *Diabetes Care*. **28**:2832–2838.

Liu, B. H., Kuo, C. F., Wang, Y. C. and Ding, S. T. (2005). Effect of docosahexaenoic acid and arachidonic acid on the expression of adipocyte determination and differentiation-dependent factor 1 in differentiating porcine adipocytes. *J. Anim. Sci.* **83**:1516–1525.

Lovegrove, J. A., Brooks, C. N., Murphy, M. C., Gould, B. J. and Williams, C. M. (1997). Use of manufactured foods enriched with fish oils as a means of increasing long-chain *n*-3 polyunsaturated fatty acid intake. *Brit. J. Nutr*. **78**:223–236.

Luff, J. (2007). Omega-3 and micro-encapsulation technology – Making functional foods taste better for longer. *Food Sci. Technolo.* **21**(1):30–31.

Lunn, J. and Buttriss, J. L. (2008). Incorporating omega-3 in the food chain—why, where and how?. *Nutr. Bull.* **33**:250–256.

Maeda, H., Hosokawa, M., Sashima, T. and Miyashita, K. (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-A^y mice. *J. Agric. Food Chem*. **55**:7701–7706.

Mai, V. and Draganov, P. V. (2009). Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health. *World J. Gastroentero.* **15**(1):81–85.

Meadus, W. J., MacInnis, R. and Dugan, M. E. (2002). Prolonged dietary treatment with conjugated linoleic acid stimulates porcine muscle peroxisome proliferator activated receptor gamma and glutamine-fructose aminotransferase gene expression *in vivo*. *J. Mol. Endocrinol*. **28**:79–86.

Melanson, E. L., Astrup, A. and Danahoo, W. (2009). The relationship between dietary fat and fatty acid intake and body weight, diabetes, and the metabolic syndrome. *Ann. Nutr. Metab.* **55**:229–243.

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Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V. and Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physico-chemical characteristics of selected vegetables. *J. Agric. Food Chem.* **56**:139–147.

Miller, K. B., Hurst, W. J., Payne, M. J., Stuart, D. A., Apgar, J., Sweigart, D. S. and Ou, B. (2008). Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *J. Agric. Food Chem.* **56**:8527–8533.

Moghadasian, M. H. (2008). Advances in dietary enrichment with N-3 fatty acids. *Crit. Rev. Food Sci. Nutr.* **48**(5):402–410.

Moran, T. H. and Dailey, M. J. (2009). Minireview: Gut peptides: Targets for antiobesity drug development? *Endocrinology*. **150**(6):2526–2530.

Moran, T. H., Smedh, U., Kinzig, K. P., Scott, K. A., Knipp, S. and Ladenheim, E. E. (2005). Peptide YY (3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**(2):R384–R388.

Munro, I. C., Harwood, M., Hlywka, J. J., Stephen, A. M., Doull, J., Flamm, W. G. and Adlerereutz, H. (2003). Soy isoflavones: A safety review. *Nutr. Rev.* **61**:1–33.

Murakami, K., Sasaki, S., Okubo, H., Takahashi, Y., Hosoi, Y. and Itabashi, M. (2007). Dietary fiber intake, dietary glycemic index and load, and body mass index: A cross-sectional study of 3931 Japanese women aged 18–20 years. *Eur. J. Clin. Nutr.* **61**:986–995.

Murase, T., Nagasawa, A., Suzuki, J., Hase, T. and Tokimitsu, I. (2002). Beneficial effects of tea catechins on diet-induced obesity: Stimulation of lipid catabolism in the liver. *Int. J. Obesity.* **26**:1459–1464.

Nagao, K. and Yanagita, T. (2005). Conjugated fatty acids in food and their health benefits. *J. Biosci. Bioeng.* **100**(2):152–157.

Nakagawa, K., Kishida, H., Arai, N., Nishiyama, T. and Mae, T. (2004). Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A^y mice. *Biol. Pharm. Bull.* **27**(11):1775–1778.

Napier, J. A. and Sayanova, A. (2005). The production of very-long-chain PUFA biosynthesis in transgenic plants: Towards a sustainable source of fish oils. *P. Nutr. Soc.* **64**:387–393.

Neary, M. T. and Batterham, R. L. (2009). Gut hormones: Implication for the treatment of obesity. *Pharmacol. Ther.* **124**:44–56.

Nielsen, N. S. and Jacobsen, C. (2009). Methods for reducing lipid oxidation in fish-oil-enriched energy bars. *Internat. J. Food Sci. Technol.* **44**:412–420.

Nielsen, N. S., Klein, A. and Jacobsen, C. (2009). Effect of ingredients on oxidative stability of fish oil-enriched drinking yogurt. *Eur. J. Lipid Sci. Tech.* **111**:337–345.

Okada, T., Mizuno, Y., Sibayama, S., Hosokawa, M. and Miyashita, K. (2011). Antiobesity effects of *Undaria* lipid capsules prepared with scallop phospholipids. *J. Food Sci*. In press. doi: 10.1111/j.1750-3841.2010.01878.x

Okuda, H. and Han, L.-K. (2001). Medicinal plant and its related metabolic modulators. *Folia Pharmacol. Jpn.* **118**(5):347–351.

Owuor, P. O. (2003). Tea: Chemistry. **In**: Encyclopaedia of Food Science and Nutrition, 2nd ed., pp: 5743–5752. Benjamin Caballero, Ed., Elsevier Science, London.

Padwal, R., Li, S. K. and Lau, D. C. W. (2003). Long-term pharmacotherapy for overweight and obesity: A systematic review and meta-analysis of randomized controlled trials. *Int. J. Obesity.* **27**:1437–1446.

Padwal, R. S. and Majumdar, S. R. (2007). Drug treatments for obesity: Orlistat, sibutramine, and rimonabant. *Lancet*. **369**:71–77.

Pereira, M. A. and Ludwig, D. S. (2001). Dietary fiber and body-weight regulation: Observations and mechanisms. *Pediatr. Clin. North Am.* **48**:969–980.

Picó, C., Oliver, P., Priego, T., Sánchez, J. and Palou, A. (2006). Functional foods and obesity: Strategies, effectiveness and safety. *Rev. Esp. Obes.* **4**(3):156–174.

Porcella, M. I., Sánchez, G., Vaudagna, S. R., Zanelli, M. L., Descalzo, A. M., Meichtri, L. H., Gallinger, M. M. and Lasta, J. A. (2001). Soy protein isolate added to vacuum-packaged *chorizos*: Effect on drip loss, quality characteristics and stability during refrigerated storage. *Meat Sci*. **57**: 437–443.

Puupponen-Pimiä, R., Aura, A.-M., Oksman-Caldentey, K.-M., Myllärinen, P., Saarela, M., Mattila-Sandhom, T. and Poutanen, K. (2002). Development of functional ingredients for gut health. *Trends Food Sci. Tech.* **13**:3–11.

Quiles-Izquierdo, J., Pérez-Rodrigo, C., Serra-Majem, L., Román, B. and Aranceta, J. (2008). Obesity in Spain: Present situation and intervention strategies. *Rev. Esp. Nutr. Comunitaria.* **14**(3):142–149.

Reimer, R. A., Pelletier, X., Carabin, I. G., Lyon, M., Gahler, R., Parnell, J. A. and Wood, S. (2010). Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur. J. Clin. Nutr.* **64**:1186–1191.

Rideout, T. C., Harding, S. V. and Jones, P. J. H. (2010). Consumption of plant sterols reduces plasma and hepatic triglycerides and modulates the expression of lipid regulatory genes and *de novo* lipogenesis in C57BL/6J mice. *Mol. Nutr. Food Res*. **54**:S7–S13.

Rincón-León, F. (2003). Functional Foods. **In**: Encyclopaedia of Food Science and Nutrition, 2nd ed., pp: 2827–2832. Benjamin Caballero, ed., Elsevier Science, London.

Rodríguez, R., Jiménez, A., Fernández-Bolaños, J., Guillén, R. and Heredia, A. (2006). Dietary fibre from vegetable products as a source of functional ingredients. *Trends Food Sci. Tech.* **17**:3–15.

Rossi, E. A., Cavallini, D. C. V., Carlos, I. Z., Vendramini, R. C., Dâmaso, A. R. and de Valdez, G. F. (2008). Intake of isoflavone-supplemented soy yogurt fermented with *Enterococcus faecium* lowers serum total cholesterol and non-HDL cholesterol of hypercholesterolemic rats. *Eur. Food Res. Technol*. **228**:275–282.

Ruiz-Rodriguez, A., Marín, F. R., Ocaña, A. and Soler-Rivas, C. (2008). Effect of domestic processing on bioactive compounds. *Phytochem. Rev.* **7**:345–384.

Saito, S., Tomonobu, K., Kudo, N., Shiiba, D., Hase, T. and Tokimitsu, I. (2006). Serum retinol, α-tocopherol, and β-carotene levels are not altered by excess ingestion of diacylglycerol-containing plant sterol esters. *Ann. Nutr. Metab.* **50**:372–379.

Sánchez-Zapata, E., Fernández-López, J., Sendra, E., Sayas-Barberá, M. E., Navarro, C., Viuda-Martos, M. and Pérez-Alvarez, J. A. (2009). Application of orange fibre for the control of oxidation in paté from yellowfin (*Thunnus albacares*) dark muscle (“sangacho”). *Alimentaria*. **400**:95.

Sehat, N., Yurawecz, M. P., Roach, J. A. G., Mossoba, M. M., Kramer, J. K. G. and Ku, Y. (1998). Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. *Lipids*. **33**:217–221.

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E., Pérez-Alvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiol*. **25**(1):13–21.

Serfert, Y., Drusch, S. and Schawarz, K. (2009). Chemical stabilisation of oils rich in long-chain polyunsaturated fatty acids during homogenization, microencapsulation and storage. *Food Chem*. **113**(4):1106–1112.

Serrano, J. and Sánchez González, I. (2008). Trends in functional foods against obesity: Functional ingredients, technologically modified foods and full diets. *Rev. Esp. Nutr. Comunitaria.* **14**(3):193–200.

Shai, I., Schwarzfuchs, D., Henkin, Y., Shahar, D. R., Witkow, S., Greenberg, I., Golan, R., Fraser, D., Bolotin, A., Vardi, H., Tangi-Rozental, O., Zuk-Ramot, R., Sarusi, B., Brickner, D., Schwartz, Z., Sheiner, E., Marko, R., Katorza, E., Thiery, J., Fiedler, G. M., Blüher, M., Stumvoll, M. and Stampfer, M. J. (2008). Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *New Engl. J. Med.* **359**(3):229–241.

Sherwood, N. E. and Story, M. (2003). Obesity: Epidemiology. **In**: Encyclopaedia of Food Science and Nutrition, 2nd ed., pp. 4241–4246. Benjamin Caballero, Ed., Elsevier Science, London.

Shi, H., DiRienzo, D. and Zemel, M. B. (2001). Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation energy-restricted aP2-agouti transgenic mice. *FASEB J*. **15**:291–293.

Shi, H., Norman, A. W., Okamura, W. H., Sen, A. and Zemel, M. B. (2002). 1,25-dihydroxyvitamin D3 inhibits uncoupling protein 2 expression in human adipocytes. *FASEB J*. **16**:1808–1810.

Shireman, R. (2003). Essential fatty acids. **In**: Encyclopaedia of Food Science and Nutrition, 2nd ed., pp: 2169–2176. Benjamin Caballero, Ed., Elsevier Science, London.

Silveira, M.-B., Carraro, R., Monereo, S. and Tébar, J. (2007). Conjugated linoleic acid (CLA) and obesity. *Public Health Nutr*. **10**(10A):1181–1186.

Slavin, J. L. (2005). Dietary fiber and body weight. *Nutrition*. **21**:411–418.

Downloaded by [Universitario Miguel Hernandez] at 04:23 12 July 2013

Smedman, A. and Vessby, B. (2001). Conjugated linoleic acid supplementation in humans-metabolic effects. *J. Nutr.* **36**:773–781.

Smith, T. H., Sim-Selley, L. J. and Selley, D. E. (2010). Cannabinoid CB_1 receptor-interacting proteins: Novel targets for central nervous system drug discovery?. *Brit. J. Pharmacol.* **160**:454–466.

Stevens, J., Ahn, K., Juhaeri, Houston, D., Steffan, L. and Couper, D. (2002). Dietary fiber intake and glycemic index and incidence of diabetes in African-American and white adults: The ARIC study. *Diabetes Care*. **25**: 1715–1721.

Sun, X. and Zemel, M. B. (2004). Role of uncoupling protein 2 (UCP2) expression and 1α, 25-dihydroxyvitamin D3 in modulating adipocyte apoptosis. *FASEB J*. **18**(12):1430–1432.

Suzuki, K., Konno, R., Shimizu, T., Nagashima, T. and Kimura, A. (2007). A fermentation product of phytosterol including campestenone reduces body fat storage and body weight gain in mice. *J. Nutr. Sci. Vitaminol.* **53**:63–67.

Takeshita, M., Saito, S., Moriwaki, J., Takase, N., Shiiba, D., Kudo, N., Nakajima, Y. and Tokimitsu, I. (2007). Effects of dietary diacylglycerol oil containing phytosterols in mayonnaise on abdominal fat and blood cholesterol levels in Japanese men. *Jpn. Pharmacol. Ther.* **39**:973–987.

Thane, C. W., Stephen, A. M. and Jebb, S. A. (2007). Whole grains and adiposity: Little association among British adults. *Eur. J. Clin. Nutr.* **63**:229–237.

Thom, E., Wadstein, J. and Gudmundson, O. (2001). Conjugated linoleic acid reduces body fat in healthy exercising humans. *J. Int. Med. Res.* **29**: 392–396.

Tsuda, T. (2008). Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. *J. Agric. Food Chem.* **56**:642–646.

Tsuda, T., Horio, F., Uchida, K., Aoki, H. and Osawa, T. (2003). Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorated hyperglycemia in mice. *J. Nutr.* **133**:2125–2130.

Tsuda, T., Ueno, Y., Kojo, H., Yoshikawa, T. and Osawa, T. (2005). Gene expression profile of isolated rat adipocytes treated with anthocyanins. *Biochim. Biophys. Acta.* **1733**:137–147.

Tsunoda, N., Ikemoto, S., Takahashi, M., Maruyama, K., Watanabe, H., Goto, N. and Ezaki, O. (1998). High-monounsaturated fat diet-induced obesity and diabetes in C57BL/6J mice. *Metabolism*. **47**:724–730.

Tsuzuki, T., Kawakami, Y., Nakagawa, K. and Miyazawa, T. (2006). Conjugated docosahexaenoic acid inhibits lipid accumulation in rats. *J. Nutr. Biochem.* **17**(8):518–524.

Tsuzuki, T., Kawakami, Y., Suzuki, Y., Abe, R., Nakagawa, K. and Miyazawa, T. (2005). Intake of conjugated eicosapentaenoic acid suppresses lipid accumulation in liver and epididymal adipose tissue in rats. *Lipids*. **40**(11):1117–1123.

Ubbink, J. and Krüger, J. (2006). Physical approaches for the delivery of active ingredients in foods. *Trends Food Sci. Tech.* **17**:244–254.

van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie, S., Somoza, V., Knorr, D., Jasti, P. R. and Eisenbrand, G. (2010). A review on the beneficial aspects of food processing. *Mol. Nutr. Food Res.* **54**:1215–1247.

Van Gaal, L. F., Rissanen, A. M., Scheen, A. J., Ziegler, O. and Rössner, S. (2005). Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*. **365**:1389–1397.

Velasquez, M. T. and Bhathena, S. J. (2007). Role of dietary soy protein in obesity. *Int. J. Med. Sci.* **4**:72–82.

Vincent, R. P. and le Roux, C. W. (2007). New agents in development for the management of obesity. *Int. J. Clin. Pract.* **61**(12):2103–2112.

Viuda-Martos, M., López-Marcos, M. C., Fernández-López, J., Sendra, E., López-Vargas, J. H. and Pérez-Álvarez, J. A. (2010a). Role of fiber in cardiovascular diseases: A review. *Compr. Rev. Food. Sci. Food Saf.* **9**:240–258.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. and Pérez-Álvarez, J. A. (2009). Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. *Innov. Food Sci. Emerg.* **10**:655–660.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. and Pérez-Álvarez, J. A. (2010b). Effect of adding citrus fibre washing water and rosemary essential oil on the quality characteristics of a bologna sausage. *LWT-Food Sci. Technol.* **43**:958–963.

Vögler, O., López-Bellan, A., Alemany, R., Tofé, S., González, M., Quevedo, J., Pereg, V., Barceló, J. and Escribá, P. V. (2008). Structure-effect relation of C18 long-chain fatty acids in the reduction of body weight in rats. *Int. J. Obesity.* **32**:464–473.

Wachtel-Galor, S., Wing Wong, K. and Benzie, I. F. F. (2008). The effect of cooking on Brassica vegetables. *Food Chem.* **110**:706–710.

Wang, Y., Jacome-Sosa, M. M., Ruth, M. R., Goruk, S. D., Reaney, M. J., Glimm, D. R., Wright, D. C., Vine, D. F., Field, C. J. and Proctor, S. D. (2009). *Trans*-11 vaccenic acid reduces hepatic lipogenesis and chylomicron secretion in JCR: LA-*cp* rats. *J. Nutr*. **139**:2049–2054.

Wang, Y., Jacome-Sosa, M. M., Vine, D. F. and Proctor, S. D. (2010). Beneficial effects of vaccenic acid on postprandial lipid metabolism and dyslipidemia: Impact of natural *trans*-fats to improve CVD risk. *Lipid Technol*. **22**(5):103–106.

Wang, Y., Lu, J., Ruth, M. R., Goruk, S. D., Reaney, M. J., Glimm, D. R., Vine, D. F., Field, C. J. and Proctor, S. D. (2008). *Trans*-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR: LA-cp rats. *J. Nutr*. **138**:2117–2122.

Westerterp-Plantenga, M. S., Lejeune, M. P. and Kovacs, E. M. (2005). Body weight loss and weight maintenance in relation to habitual caffeine intake and green tea supplementation. *Obes. Res.* **13**:1195–1204.

WHO, World Health Organization. (1998). Obesity: Preventing and managing the global epidemic. WHO technical report series 894. *Geneva*.

WHO, World Health Organization. (2003). Diet, nutrition and the prevention of chronic diseases. WHO technical report series 916. *Geneva*.

WHO, World Health Organization. (2009). Available from http://www.who.int/healthinfo/global_burden_disease/global_health_risks/en/index.html

WHO, World Health Organization. (2010). Available from http://www.who.int/features/factfiles/obesity/facts/en/index9.html. Consulted January 11th 2011.

Willumsen, N., Hexeberg, S., Skorve, J., Lundquist, M. and Berge, R. K. (1993). Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. *J. Lipid Res.* **34**:13–22.

Wolfram, S., Wang, Y., Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Mol. Nutr. Food Res.* **50**:176–187.

Wu, J., Oka, J., Higuchi, M., Tabata, I., Toda, T., Fujioka, M., Fuku, N., Teramoto, T., Okuhira, T., Ueno, T., Uchiyama, S., Urata, K., Yamada, K. and Ishimi, Y. (2006). Cooperative effects of isoflavones and exercise on bone and lipid metabolism in postmenopausal Japanese women: A randomized placebo-controlled trial. *Metabolism*. **55**:423–433.

Wu, C.-H., Yang, M.-Y., Chan, K.-C., Chung, P.-J., Ou, T.-T. and Wang, C.-J. (2010). Improvement in high-fat diet-induced obesity and body fat accumulation by a *Nelumbo nucifera* leaf flavonoid-rich extract in mice. *J. Agric. Food Chem*. **58**:7075–7081.

Yep, Y. L., Li, D., Mann, N. J., Bode, O. and Sinclair, A. J. (2002). Bread enriched with microencapsulated tuna oil increases plasma docosahexaenoic acid and total omega-3 oils in humans. *Asia Pac. J. Clin. Nutr.* **11**(4):285–291.

Zemel, M. B. (2002). Regulation of adiposity and obesity risk by dietary calcium: Mechanisms and implications. *J. Am. Coll. Nutr.* **21**(2):146S–151S.

Zemel, M. B., Shi, H., Greer, B., DiRienzo, D. and Zemel, P. C. (2000). Regulation of adiposity by dietary calcium. *FASEB J*. **14**:1132–1138.

Zemel, M. B. (2003). Mechanisms of dairy modulation of adiposity. *J. Nutr.* **133**:252S–256S.

Zemel, M. B. (2005). The role of dairy foods in weight management. *J. Am. Coll. Nutr.* **24**(6):537S–546S.

Zhao, H. L., Harding, S. V., Marinangeli, C. P. F., Kim, Y. S. and Jones, P. J. H. (2008). Hypocholesterolemic and anti-obesity effects of saponins from *Platycodon grandiflorum* in hamsters fed atherogenic diets. *J. Food Sci.* **73**(8):195–200.

Zheng, G., Sayama, K., Okubo, T., Juneja, L. R. and Oguni, I. (2004). Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. *In Vivo*. **18**:55–62.

7.2. PUBLICACIONES EN REVISIÓN

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PUBLICACIÓN 7

Fatty acid and conjugated linoleic acid (CLA) content in fermented milks as assessed by direct methylation

Lorena Trigueros, Esther Sendra

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Article Type: Research Article

Keywords: dairy products, fatty acid profile, conjugated linoleic acid, direct methylation

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Abstract: It has been investigated the CLA content and fatty acid profile of several commercial Spanish plain fermented milks (FM) by direct methylation. Direct methylation avoids extraction procedures and so reduces solvent consumption, being cost efficient and environmentally friendly, whereas free of interferences due to solvent affinity. Regular yogurt and FM containing probiotic bacteria were investigated. High-fat, full-fat and low-fat types were tested (samples included FM with probiotic bacteria and FM with regular yogurt cultures). CLA content in FM ranged from non-detected to 93.33 mg/100g. Neither the fat content of FM nor the presence of probiotics affected the CLA content (% of total fatty acids). Regarding CLA content per serving size of FM only fat content was positively correlated with CLA.

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Campus de Orihuela

Orihuela, March 5th, 2014

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Eidgenössische Technische Hochschule (ETH) Zürich, Schmelzbergstrasse 9, 8092 Zürich, Switzerland

Dear Dr. Hecht,

Please consider the attached manuscript to be submitted for publication in the Food Chemistry.

1. Manuscript title: FATTY ACID AND CONJUGATED LINOLEIC ACID (CLA) CONTENT IN FERMENTED MILKS AS ASSESED BY DIRECT METHYLATION
2. Corresponding author: Esther Sendra, Departamento de Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández. Ctra. Beniel km 3,2, 03312 Orihuela (Alicante)-SPAIN. Phone: +34966749735, Fax: +34966749677.

Topic: The present work is a contribution to the study of the presence of CLA in widely consumed fermented milks in Spain. Analyses were performed by direct extraction methylation which is a rapid, simple and reliable method. Results showed that the fat level of the samples tested did not affect CLA content. Also the presence of probiotic cultures was not a significant factor.

Please do not hesitate to contact me in case you need any further information. We thank you very much for your assistance.

Yours sincerely,

Dra. Esther Sendra Nadal
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*Highlights (for review)

1 • Individual isomers of CLA may contribute in different ways in health
2 effects.
3 • Direct extraction methylation was used to determine CLA in fermented
4 milks.
5 • CLA content in comercial fermented milks ranged from non-detected to
6 93.3 mg/100 g.
7 • Average contribution of CLA in the total fatty acid profile accounted for
8 0.83%.
9 • The presence of probiotic bacteria did not affect CLA content.

*Manuscript
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1 **FATTY ACID AND CONJUGATED LINOLEIC ACID (CLA) CONTENT IN**
2 **FERMENTED MILKS AS ASSESSED BY DIRECT METHYLATION**
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UNIVERSITAS Miguel Hernández

15 **Abstract**

16 It has been investigated the CLA content and fatty acid profile of several
17 commercial Spanish plain fermented milks (FM) by direct methylation. Direct
18 methylation avoids extraction procedures and so reduces solvent consumption,
19 being cost efficient and environmentally friendly, whereas free of interferences
20 due to solvent affinity. Regular yogurt and FM containing probiotic bacteria were
21 investigated. High-fat, full-fat and low-fat types were tested (samples included
22 FM with probiotic bacteria and FM with regular yogurt cultures). CLA content in
23 FM ranged from non-detected to 93.33 mg/100g. Neither the fat content of FM
24 nor the presence of probiotics affected the CLA content (% of total fatty acids).
25 Regarding CLA content per serving size of FM only fat content was positively
26 correlated with CLA.

27

28 **Key words:** dairy products, gas chromatography, fatty acid profile, conjugated
29 linoleic acid, probiotic, direct methylation

30

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32 **1. Introduction**

33 Conjugated linoleic acid (CLA), a natural derivative of linoleic acid (LA),
34 has attracted strong interest due to its potentially beneficial biologic effects of
35 attenuating lifestyle-related diseases (Dilzer & Park, 2012). CLA is a term used
36 to describe a heterogeneous group of positional and geometric isomers of LA
37 (*cis*-9, *cis*-12 C18:2) with conjugated double bonds (Andrade et al., 2012).
38 Evidence has suggested that individual isomers may contribute in different ways
39 in their beneficial or potential side effects (Rodríguez-Alcalá & Fontecha, 2007).
40 The *cis*-9, *trans*-11 CLA isomer (c9 t11 CLA, denominated Rumenic acid (RA))
41 is the major and most active isomer of CLA and may represent up to 80% of the
42 total CLA in food. Although found naturally at low levels, the other main isomer
43 of CLA, *trans*-10, *cis*-12 CLA (t10, c12 CLA), is present in significant amounts
44 from synthetically prepared CLA which has been extensively used for most
45 animal and human studies (Andrade et al., 2012; Dilzer & Park, 2012). RA is
46 responsible for the anticarcinogenic properties of CLA as well as growth-
47 promoting and antiatherogenic effects, whereas the t10, c12 CLA is responsible
48 of the antiobese effect (Rodríguez-Alcalá & Fontecha, 2007).

49 Meat and dairy products from ruminant animals (such as milk, butter,
50 yogurt and cheese) are the principal natural sources of CLA in the human diet.
51 Contents of CLA in dairy products range between 3.3 to 8 mg/g of fat (Lin,
52 Boylston, Chang, Luedecke, & Shultz, 1995). The fact that dairy products have
53 been identified as good sources of CLA could increase the positive nutritional
54 image of these foods. Several studies have proved that fermented dairy
55 products contain higher levels of CLA than non-fermented milk (Santha, Ram,
56 O'leary, Hicks, & Decker, 1995; Yadav, Jain, & Sinha, 2007). Few data is

available on the naturally occurring CLA content in commercial fermented milks, and no data as assessed by direct methylation.

The fatty acid (FA) content in a lipid extract is usually determined by gas chromatography and transformation of FA into FA methyl ester (FAME) is required. FAME can be prepared either by multistep methods involving lipid extraction followed by transmethylation, or by direct methylation (DM). DM presented several advantages over the extraction-based methods, such as small samples sizes, low solvent consumption and high recovery for polar lipids that are tightly bound to the matrix (Xiao, Mjøs, & Haugsgjerd, 2012). Several studies have validated the efficacy of the DM methodology in milk matrices showing high recoveries and a constant reproducibility for FA in general (Cantellops, Reid, Eitenmiller, & Long, 1999; Löpez-Löpez, Castellote-Bargalló, & López-Sabater, 2001) and CLA in particular (Moltó-Puigmartí, Castellote, & López-Sabater, 2007). However in other food matrices, as eggs, contradictory results were found when DM was used (Wang, Sunwoo, Cherian, & Sim, 2000; Mazalli & Bragagnolo, 2007).

The aim of this work is to determine the fatty acid profile and the CLA content by direct extraction methylation of widely consumed fermented milks with different fat contents and to detect whether the presence of probiotics in the starter culture used affects the mentioned CLA content in commercial fermented milks.

2. Materials and methods

2.1. Materials

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81 All reagents used in the lab procedure were GC grade: hexane,
82 methanol, boron triflouride (BF_3) were obtained from Sigma-Aldrich Chemie
83 GmbH (Steinheim, Germany); methylene chloride from Labscan, Ltd (Dublin,
84 Ireland); sodium hydroxide and anhydrus sodium sulphate were from Panreac
85 (Castellar del Vallès, Barcelona, Spain).

86 Fatty acid methyl ester (FAME) Mix Supelco GLC-50, GLC-100, CLA
87 isomers (c9 t11 CLA and t10 c12 CLA), nonanoic acid (C9:0) and
88 heptadecanoic acid (C17:0) were purchased from Sigma-Aldrich Chemie GmbH
89 (Steinheim, Germany).

90 **2.2. Samples**

91 Twenty-four commercially available plain fermented milks (FM) (from cow
92 milk) were purchased in triplicate from different local supermarkets in Spain and
93 transported to the laboratory at 4°C, according to labeling information: four full-
94 fat (Y1, Y2, Y3, Y4) and three low-fat (Y1-L, Y2-L, Y3-L) FM made with regular
95 yogurt cultures (RYC) (*Lactobacillus delbrueckii* subsp. *bulgaricus* and
96 *Streptococcus thermophilus*), four high-fat (G1, G2, G3, G4) and one full-fat
97 (G1-L) Greek style FM made with RYC, three full-fat (B1, B2, B3) and three low-
98 fat (B1-L, B2-L, B3-L) commercial probiotic FM made with RYC and
99 *Bifidobacterium* spp., three full-fat (Lc1, Lc2, Lc3) and three low-fat (Lc1-L, Lc2-
100 L, Lc3-L) commercial probiotic FM made with RYC and *L. casei*. Table 1
101 summarizes main product information of the FM based on labelling information.
102 Samples were maintained at this temperature until pH and FAMEs analysis.

103 **2.3. pH**

104 pH was determined using a pH-meter (GLP21, Crison Instruments,
105 Barcelona, Spain).

106 **2.4. Lipid analysis**

107 2.4.1. Methylation procedure

108 Fatty acids were *in situ* methylated according to the Park & Goins (1994)
109 method with some modifications. Basically, 150 µL of high and full-fat or 250 µL
110 of low-fat FM were transferred into a test tube with 40 µL of C9:0 and 80 µL of
111 C17:0 *n*-hexane solutions (20 mg/mL) as internal standards. Then, 100 µL of
112 methylene chloride and 1 mL of 0.5N NaOH in methanol were added and the
113 tubes were heated in a water bath at 90°C for 10 min. One mL of BF_3 in
114 methanol was added and the mixture was left at room temperature (25°C) for 30
115 min to prevent intraisomerization of CLA isomers (Werner, Luedecke, & Shultz,
116 1992). One mL of distilled water and 600 µL hexane were added and then
117 FAMEs were extracted by vigorous shaking for about 1 min. Following
118 centrifugation, the aliquots were dried with anhydrus sodium sulphate and the
119 top layer was transferred into a vial flushed with nitrogen which was stored at -
120 20°C until analyzed by gas chromatography.

121 2.4.2. Gas chromatography (GC) analysis

122 The fatty acid composition of FAMEs was analyzed on an Agilent gas
123 chromatography unit (model 6890, Palo Alto, CA, USA) equipped with a flame
124 ionization detector (FID) and a DB-23 capillary column (30 m length, 0.25 µm
125 film, 0.25 mm internal diameter; J&W Scientific, Agilent Technologies). The flow
126 rate of the carrier gas (Helium) was 1.2 mL/min and 35 mL/min at the make-up
127 point, the injector temperature was 200°C and the detector 220°C. The injection
128 volume was 0.5 µL (splitless). The temperature program was as follows: initial
129 temperature 70°C, temperature from 70 to 190°C at 10°C/min for 20 min, from
130 190 to 240°C at 5°C/min and at 240°C held isothermally for 3 min.

Identification of the FAME peaks was performed by comparing the retention times of the FAME standards. Agilent technologies software (G2072AA Rev.A.05.02 Chemstation) was used for integration of peaks. CLA and fatty acid concentrations in fermented milks were expressed as percentage of total fatty acids using response factors which were previously determined.

2.5. Statistical analysis

Three batches of each fermented milk were analyzed. General Linear Model procedure was used to evaluate fat content (high, >8%; full, 1.4-4%; low, <0.5%) and probiotic presence (probiotic (B or Lc) and no probiotic) in the starter culture on the evaluated parameters (SPSS statistical software, version 20.0, Chicago, IL, USA), using Tukey's pairwise comparisions post-hoc test with a significance level of 0.05.

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3. Results and discussion

3.1. pH

pH of FM manufactured with TYC which includes Y-types and G-types ranged between 4.05 and 4.46. For probiotic FM made with TYC and *Bifidobacteria* spp. pH ranged between 4.02 and 4.36. pH of probiotic FM made with TYC and *L. casei* ranged between 3.86 and 4.38. Similar pH range was observed for all FM.

3.2. Fatty acid composition of high-fat and full-fat fermented milks

Based on previous experience on DM, and as pointed out in the materials and methods section, different aliquot volumes were taken for fat free and full fat FM. Given the extremely low fat content of fat free dairy products if smaller volumes are taken even major fatty acids may have not been detected

156 by GC analysis. 250 microliters allowed the detection of major fatty acids and
157 good reproducibility of results in fat free milk (coefficient of variation under 2%).
158 Average fatty acid composition for high and full fat fermented milks analyzed
159 ranged as follows (% of total fatty acids): short chain fatty acids C6:0 from
160 1.29% to 1.65%, C8:0 from 1.14% to 1.36%, C10:0 from 2.81% to 3.54%;
161 medium chain fatty acids C12:0 from 3.34% to 4.34%, C14:0 from 11.86% to
162 14.59%, C15:0 from 1.05% to 1.44%; long chain fatty acids *cis*-6, *cis*-9, *cis*-12
163 C18:3 (gamma-Linolenic acid) from 0.06% to 0.21%, C19:0 from non-detected
164 to 0.14%, *cis*-9, *cis*-12, *cis*-15 C18:3 (alpha-Linolenic acid) from 0.40% to
165 0.99%, C20:0 from 0.07% to 0.17%, C21:0 from 0.06% to 0.21% and C20:5
166 from non-detected to 0.44%. Table 2 contains data on main long chain fatty
167 acids C16, C16:1, C18:0, C18:1, C18:2, c9 t11 CLA and t10 c12 CLA in the
168 studied commercial fermented milks. The fatty acid profile of low fat yogurts
169 resulted in several non-detected peaks due to a fat content below 0.1%. Factors
170 associated with the animal such as genetics, stage of lactation, ruminal
171 fermentations or the feed such as grain intake, amount and composition of
172 dietary fat, seasonal and regional effects influence the amount and fatty acid
173 composition of bovine milk lipids (Jensen, 2002).

174 The average composition of fatty acids as well as their distribution in the
175 saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids
176 fraction were in accordance with the reported values reviewed by Jensen
177 (2002). As expected, the most prevalent fatty acid fraction corresponded to SFA
178 with 68.87% on average and the G4 FM presenting the highest relative content
179 (70.09%). The main fatty acids in the SFA fraction were Palmitic acid (C16:0)
180 followed by Stearic acid (C18:0) for all samples, independently of the FM type,

181 with an average relative content of 32.09% and 12.01%, respectively.
182 Regarding the unsaturated fatty acid fraction, the major fatty acid was Oleic acid
183 (C18:1) with an average relative concentration of 25.30%. Palmitoleic acid
184 (C16:1) was the following fatty acid among MUFA, with 1.52% on average. The
185 total relative content of PUFA fraction was 4.31% on average, being the Y1 the
186 FM which presented the highest relative content (5.87%). Linoleic acid (C18:2)
187 composed the majority of the PUFA fraction with an average content of 2.71%
188 followed by Linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3) with 0.52%. Lc2 FM
189 presented the lowest CLA content (0.73%) and G2 presented the highest one
190 (1.18%). In the present study the contribution of CLA in the total fatty acids
191 accounted for 0.83% on average. The CLA values obtained were well supported
192 by prior research reports for FM (Fritsche & Steinhart, 1998; Serafeimidou,
193 Zlatanos, Laskaridis, & Sagredos, 2012).

194 **3.3. CLA content in the fatty acid profile: CLA content of high-fat, full-fat**
195 **and low-fat fermented milks**

196 As we can see in Table 2 in the majority of samples analyzed the major
197 isomer determined was the c9 t11 CLA. Some of the FM analyzed presented
198 the t10 c12 CLA isomer as the predominant one, being all of them low-fat type.
199 This result may be irrelevant due to the low fat content of low-fat FM and the
200 difficulties in detection of minority fatty acids in such samples. The CLA content
201 of dairy products is dependent on the initial CLA content of the raw milk, formed
202 through microbial enzymatic reactions in the rumen and further isomerization
203 reactions during processing (Serafeimidou, Zlatanos, Laskaridis, & Sagredos,
204 2012). The CLA content (as the average of each FM type) in the FM was 0.76%
205 for B, 0.84% for Lc, 0.81% for Y, 0.91% for G, 0.77% for B-L, 0.78% for Lc-L,

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0.95% for Y-L and 0.64% for G-L. As the average total CLA content in raw cow milk was 0.56% (of total fatty acids) (Rodríguez-Alcalá, Harte, & Fontecha, 2009) we may conclude that the fermentation process affects CLA content. These results were in accordance with previous reports where the fermented product presented higher levels of CLA than their non-fermented counterparts: in *dahi* (Aneja & Murthy, 1990), and in yoghurt (Santha, Ram, O'Learly, Hicks, & Decker, 1995). The contribution of c9 t11 CLA and the t10 c12 CLA in the total fatty acids accounted for 0.50% and 0.33% on average, respectively. The concentration of the c9 t11 CLA isomer is similar to the reported in previous papers. Serafeimidou, Zlatanos, Laskaridis, & Sagredos, (2012) found concentrations between 1 to 45 mg/100 g sample in traditional Greek yogurts made from cow milk (3.3% fat on average). In the study carried out by Lin (1995) yogurts (1.9% fat content) presented 7 mg/100g sample of the mentioned CLA isomer. In the present study FM with a fat content of 3% presented concentrations of c9 t11 CLA isomer ranged between 7.42 to 18.78 mg/100g sample. Total CLA content ranged from non-detected (in low-fat FM) to 93.33 mg/100 g sample.

Neither the fat percentage of the samples nor the type of the starter culture affected the total CLA content of the FM ($p>0.05$) when results are in % of total fatty acids (Table 2). Only the c9 t11 CLA isomer individually was affected, being the content significantly higher in the full-fat Greek style FM ($p<0.05$) with regard to the low-fat FM ones (all studied types). In a dairy product CLA formation is dependent on numerous factors such as bacterial strain, cell number, optimal substrate concentration and the period of incubation at neutral pH (Kim & Liu, 2002). From the labeling information we can assume

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231 that in the present study dairy products were elaborated with no additional
232 substrates for the CLA formation so solely the type of lactic acid bacteria (LAB)
233 used or the initial fat content of the milk could affect the CLA concentration.

234 **3.4. CLA content of probiotic and non-probiotic fermented milks**

235 In the present study probiotic FM did not contain significantly a different
236 CLA content compared to non probiotics. Pandit, Anand, Kalscheur, & Hassan,
237 (2012) screened a total of 155 LAB for their ability to produce CLA from
238 nonsupplemented milk with any source of LA. They identified 12 CLA positive
239 cultures being *Lactococcus lactis* the LAB which produced the highest content
240 (1.12 g/100 g fatty acid). Yadav, Luedecke, & Shultz, (2007) prepared *dahi* (an
241 Indian equivalent yogurt) and proved that only probiotic *dahi* made with *L.*
242 *acidophilus* and *L. casei* increased CLA content during fermentation by lipolysis
243 of natural milk fat. However in the study carried out by Gorissen *et al.* (2012) no
244 differences in CLA content were detected when milk was enriched with
245 vegetable oils (as a source of LA) and fermented with probiotic bacteria.

246 **3.5. CLA content per fermented milk serving size**

247 Table 3 shows the CLA and fat content of the different commercial FM
248 analyzed. CLA content was affected by the fat content of the FM when the
249 statistical evaluation was carried out in mg/100g sample. The superior fat
250 content, the higher CLA concentration ($p<0.05$). The estimated daily human
251 CLA intake ranges from 200 to 1000 mg per day (Van Wijlen & Colombani,
252 2010) but these concentrations are probably not high enough to exert the
253 potential health effects of CLA, for example Ip, Thompson, & Scimeca, (1994)
254 estimated that a 70-kg human should consume 3000 mg of CLA/d to achieve a
255 minimum health benefits. Based on these data our finding indicates that a daily

256 consumption of 1 serving size (125g) of high-fat, full-fat or low-fat FM count up
257 to 2.97%, 0.75% and 0.11% of the daily recommendations, respectively.
258

259 **4. Conclusions**

260 The direct methylation for quantitative analysis of fatty acids has
261 demonstrated to be a rapid alternative to the established extraction-based
262 methods in milk samples containing low or high fat level, allowing the
263 quantification of CLA with good reproducibility. This study indicates that the CLA
264 content on the total fatty acids profile of commercially available fermented milks
265 varied considerably from non-detected (fat free FM) to 1.46% (Greek style). The
266 statistical study revealed that neither the fat content of the different fermented
267 milks nor the starter cultures used affected the CLA content (as % of total fatty
268 acids). Only high-fat FM contained higher levels ($p<0.05$) of the c9 t11 CLA
269 isomer than the low-fat FM (all studied types). On the other hand, fat content
270 influenced CLA concentration in mg/100g FM; the superior fat content, the
271 higher CLA concentration. Regarding consumption recommendations, CLA
272 content in FM is higher than that previously reported on milk, however the
273 fortification of FM with CLA may be of great interest for the food industry to
274 enhance CLA consumption.
275

276 **References**

277 Andrade, J. C., Ascenção, K., Gullón, P., Henriques, S. M. S., Pinto, J. M. S.,
278 Rocha-Santos, T. A. P., et al. (2012). Production of conjugated linoleic
279 acid by food-grade bacteria: A review. *International Journal of Dairy*
280 *Technology, 65 (4)*, 467-481.

281 Aneja, R. P., & Murthy, T. N. (1990). Conjugated linoleic acid contents of Indian
282 curds and ghee. *Indian Journal of Dairy Science, 43*, 231-238.
283 Cantellops, D., Reid, A. P., Eitenmiller, R. R., & Long, A. R. (1999).
284 Determination of lipids in infant formula powder by direct extraction
285 methylation of lipids and fatty acid methyl esters (FAME) analysis by gas
286 chromatography. *Journal of AOAC International, 82(5)*, 1128-1139.
287 Dilzer, A., & Park, Y. (2012). Implication of conjugated linoleic acid (CLA) in
288 human health. *Critical Reviews in Food Science and Nutrition, 52*, 488-
289 513.
290 Fritsche, J., & Steinhart, H. (1998). Amounts of conjugated linoleic acid (CLA) in
291 German foods and evaluation of daily intake. *Zeitschrift fur Lebensmittel-*
292 *Untersuchung und –Forschung, 206*, 77-82.
293 Gorissen, L., Raes, K., De Smet, S., De Vuyst, L., & Leroy, F. (2012). Microbial
294 production of conjugated linoleic and linolenic acids in fermented foods:
295 Technological bottlenecks. *European Journal of Lipid Science and*
296 *Technology, 114*, 486-491.
297 Ip, C., Thompson, H. J., & Scimeca, J. A. (1994). Conjugated linoleic acid
298 suppresses mammary carcinogenesis and proliferative activity of the
299 mammary gland in the rat. *Cancer Research, 54*, 1212-1215.
300 Jensen, R. G. (2002). The composition of bovine milk lipids: January 1995 to
301 December 2000. *Journal of Dairy Science, 85*, 295-350.
302 Kim, W. J., & Liu, R. H. (2002). Increase of conjugated linoleic acid content in
303 milk by fermentation with lactic acid bacteria. *Journal of Food Science,*
304 *67 (5)*, 1731-1737.

305 Lin, H., Boylston, T. D., Chang, M. J., Luedecke, L. O., & Shultz, T. D. (1995).
306 Survey of the conjugated linoleic acid contents in dairy products. *Journal*
307 *of Dairy Science, 78*, 2358-2365.
308 Löpez-Löpez, A., Castellote-Bargalló, A. I., & López-Sabater, M. C. (2001).
309 Comparison of two direct methods for the determination of fatty acids in
310 human milk. *Chromatographia, 54*, 743-747.
311 Mazalli, M. R., & Bragagnolo, N. (2007). Validation of two methods for fatty acid
312 analysis in eggs. *Lipids, 42*, 483-490.
313 Moltó-Puigmartí, C., Castellote, A. I., & López-Sabater, M. C. (2007).
314 Conjugated linoleic acid determination in human milk by fast-gas
315 chromatography. *Analytica Chimica Acta, 602*, 122-130.
316 Pandit, A., Anand, S., Kalscheur, K., & Hassan, A. (2012). Production of
317 conjugated linoleic acid by lactic acid bacteria in milk without any
318 additional substrate. *Internatinal Journal of Dairy Technology, 65 (4)*,
319 603-608.
320 Park, P. W., & Goins, R. E. (1994). *In situ* preparation of fatty acid methyl esters
321 for analysis of fatty acid composition in foods. *Journal of Food Science,*
322 *59*, 1262-1266.
323 Rodríguez-Alcalá, L. M., Harte, F., & Fontecha, J. (2009). Fatty acid profile and
324 CLA isomers content of cow, ewe and goat milks processed by high
325 pressure homogenization. *Innovative Food Science and Emerging*
326 *Technologies, 10*, 32-36.
327 Rodríguez-Alcalá, L. M., & Fontecha, J. (2007). Fatty acid and conjugated
328 linoleic acid (CLA) isomer composition of comercial CLA-fortified dairy

329 products: evaluation after processing and storage. *Journal of Dairy*
330 *Science, 90 (5)*, 2083-2090.
331 Santha, N. C., Ram, L. N., O'leary, J., Hicks, C. L., & Decker, E. A. (1995).
332 Conjugated linoleic acid concentrations in dairy products as affected by
333 processing and storage. *Journal of Food Science, 60*, 695-720.
334 Serafeimidou, A., Zlatanos, S., Laskaridis, K., & Sagredos, A. (2012). Chemical
335 characteristics, fatty acid composition and conjugated linoleic acid (CLA)
336 content of traditional Greek yogurts. *Food Chemistry, 134*, 1839-1846.
337 van Wijlen, R. P. J., & Colombani, P. C. (2010). Grass-based ruminant
338 production methods and human bioconversion of vaccenic acid with
339 estimations of maximal dietary intake of conjugated linoleic acid.
340 *International Dairy Journal, 20*, 433-448.
341 Wang, Y., Sunwoo, H., Cherian, G., & Sim, J. S. (2000). Fatty acid
342 determination in chicken egg yolk: a comparison of different methods.
343 *Poultry Science, 79*, 1168-1171.
344 Werner, S. A., Luedecke, L. O., & Shultz, T. D. (1992). Determination of
345 conjugated linoleic acid content and isomer distribution in three cheddar-
346 type cheeses: effects of cheese cultures, processing and aging. *Journal*
347 *of Agricultural and Food Chemistry, 40*, 1817-1821.
348 Xiao, L., Mjøs, S. A., & Haugsgjerd, B. O. (2012). Efficiencies of three common
349 lipid extraction methods evaluated by calculating mass balances of the
350 fatty acids. *Journal of Food Composition and Analysis, 25*, 198-207.
351 Yadav, H., Jain, S., & Sinha, P. R. (2007). Production of free fatty acids and
352 conjugated linoleic acid in probiotic dahi containing *Lactobacillus*

353 *acidophilus* and *Lactobacillus casei* during fermentation and storage.
354 *International Dairy Journal, 17*, 1006-1010.
355

356 Table 1. Product information of the different fermented milks.

Component	Y	G	B	Lc	Y-L	G-L	B-L	Lc-L
pH	4.21	4.11	4.21	4.00	4.26	4.26	4.21	4.16
Fat (%)*	2.95	9.20	3.60	1.87	0.20	2.90	0.30	0.27
Protein (%)*	3.40	3.47	3.40	2.67	4.20	5.60	4.53	3.03
Probiotic	No	No	Yes	Yes	No	No	Yes	Yes
Serving size (g)*	125	125	125	100	125	125	125	100

357 * Information provided by the product labeling.

358 Table 2. Fatty acid composition of fermented milks (n=3, mean ± standard error).

	Fatty acids, % of total fatty acids										
FM type	C16:0	C16:1	C18:0	C18:1	C18:2	c9 t11 CLA	t10 c12 CLA	SFA	MUFA	PUFA	ΣCLA
B1	31.87±0.79	1.46±0.05	12.36±0.25	25.60±0.64	2.87±0.09	0.57±0.00	0.19±0.00	68.67±0.82	27.06±0.68	4.27±0.14	0.77±0.01
B2	32.41±0.11	1.52±0.05	11.94±0.36	24.61±0.10	2.81±0.10	0.56±0.06	0.22±0.00	69.60±0.11	26.13±0.15	4.27±0.04	0.77±0.07
B3	31.64±0.16	1.41±0.05	12.20±0.03	24.69±0.02	2.89±0.04	0.55±0.02	0.20±0.00	69.49±0.22	26.10±0.03	4.41±0.25	0.75±0.03
Lc1	32.50±0.81	1.52±0.04	12.37±0.30	25.68±0.59	2.51±0.07	0.67±0.02	0.24±0.00	68.66±0.71	27.20±0.63	4.14±0.07	0.91±0.03
Lc2	33.47±0.24	1.59±0.03	12.22±0.14	24.37±0.55	2.56±0.04	0.52±0.00	0.21±0.00	70.07±0.57	25.96±0.58	3.98±0.01	0.73±0.00
Lc3	32.15±0.23	1.52±0.02	12.01±0.45	24.60±0.48	2.43±0.08	0.63±0.02	0.25±0.00	69.93±0.60	26.12±0.50	3.96±0.11	0.88±0.03
Y1	29.07±0.74	1.26±0.01	13.63±0.42	28.29±0.98	4.00±0.23	0.59±0.01	0.23±0.01	64.58±1.65	29.55±0.97	5.87±0.69	0.82±0.02
Y2	32.87±0.58	1.59±0.01	11.78±0.22	25.22±0.98	2.64±0.10	0.56±0.00	0.21±0.01	69.02±1.11	26.81±0.99	4.17±0.12	0.78±0.01
Y3	30.54±0.50	1.31±0.00	13.07±0.08	26.43±0.22	3.26±0.04	0.60±0.01	0.24±0.00	67.17±0.23	27.74±0.23	5.09±0.49	0.84±0.01
Y4	33.43±0.72	1.64±0.02	11.42±0.17	25.57±0.04	2.47±0.16	0.59±0.02	0.20±0.00	68.84±0.11	27.21±0.06	3.95±0.05	0.79±0.03
G1	32.75±1.32	1.63±0.12	11.13±1.12	24.38±1.28	2.59±0.58	0.61±0.06	0.19±0.01	69.96±1.61	26.01±1.16	4.02±0.46	0.79±0.05
G2	30.29±0.09	1.52±0.01	11.19±0.02	25.34±0.19	2.11±0.06	0.98±0.00	0.20±0.01	68.58±0.25	26.86±0.21	4.55±0.05	1.18±0.02
G3	33.22±1.17	1.66±0.10	11.29±1.36	24.48±1.53	2.61±0.58	0.63±0.06	0.19±0.01	69.95±1.86	26.14±1.43	3.92±0.44	0.81±0.06
G4	32.62±0.07	1.63±0.01	11.31±0.04	24.61±0.13	2.21±0.02	0.63±0.00	0.21±0.00	70.09±0.15	26.24±0.12	3.67±0.03	0.84±0.00
B1-L	32.15±0.21	1.44±0.18	12.83±0.14	25.83±0.62	3.53±0.15	0.49±0.04	0.36±0.00	67.67±1.15	27.27±0.48	5.06±0.29	0.85±0.06
B2-L	30.73±0.30	1.54±0.11	13.62±0.31	23.73±0.38	6.19±0.24	ND	ND	64.85±0.24	23.73±0.32	6.42±0.13	ND
B3-L	31.15±0.11	1.59±0.04	12.82±0.25	24.17±0.24	3.32±0.18	0.50±0.11	0.96±0.02	68.98±0.18	25.76±0.41	5.26±0.24	1.46±0.04
Lc1-L	32.48±0.15	1.37±0.08	14.36±0.08	26.93±0.27	4.03±0.42	0.54±0.08	0.34±0.01	66.11±0.95	28.29±0.44	5.60±0.15	0.88±0.01
Lc2-L	30.43±0.14	1.41±0.04	16.78±0.40	21.17±0.75	4.96±0.31	ND	ND	69.09±0.97	22.58±0.65	8.32±0.35	ND
Lc3-L	30.75±0.28	1.14±0.08	21.82±0.45	19.51±0.71	4.23±0.15	ND	1.46±0.01	73.66±1.15	20.65±0.28	5.69±0.44	1.46±0.01
Y1-L	30.91±0.41	1.42±0.14	12.08±0.25	24.08±0.41	3.15±0.12	0.50±0.05	0.24±0.02	69.94±0.35	25.51±0.27	4.55±0.47	0.74±0.02
Y2-L	30.26±0.54	1.23±0.12	16.45±0.31	19.98±0.40	4.53±0.39	ND	ND	63.77±0.61	21.21±0.38	6.02±0.68	ND
Y3-L	33.03±0.19	1.11±0.20	14.03±0.14	24.59±0.84	4.72±0.24	0.75±0.02	1.35±0.02	68.59±0.64	24.59±0.39	6.82±0.14	2.11±0.01
G1-L	31.17±0.24	1.51±0.05	10.24±0.08	22.21±0.16	2.42±0.11	0.45±0.01	0.20±0.00	72.62±0.18	23.72±0.44	3.66±0.31	0.64±0.04

359 Abbreviation: not detected (ND), *trans* (t), *cis* (c), conjugated linoleic acid (CLA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA),
360 polyunsaturated fatty acids (PUFA).
361 SFA: sum of C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C19:0, C20:0 and C21:0; MUFA: sum of C16:1 and C18:1; PUFA: sum of all C18:2,
362 C18:3 and C20:5.

363 Table 3. CLA (mg/100 g sample) and fat (g/100 g sample) content of fermented

364 milks (n=3).

FM type	Total CLA (mg/100 g sample)		Fat (g/100 g sample)*
	Mean	SE	
B1	24,81	0.73	3,70
B2	22,46	2.38	3,50
B3	25,63	0.82	3,60
Lc1	22,19	2.35	2,60
Lc2	11,95	0.80	1,60
Lc3	10,42	0.27	1,40
Y1	21,70	0.71	2,90
Y2	20,87	1.32	3,60
Y3	12,82	0.56	2,20
Y4	23,44	0.60	3,10
G1	64,47	1.02	10,00
G2	93,33	1.95	8,70
G3	73,32	1.47	9,10
G4	53,78	0.27	9,00
B1-L	3,35	0.03	0,40
B2-L	ND	---	0,10
B3-L	1,64	0.04	0,40
Lc1-L	3,96	0.11	0,10
Lc2-L	ND	---	0,20
Lc3-L	1,10	0.05	0,50
Y1-L	2,94	0.14	0,40
Y2-L	ND	---	0,10
Y3-L	2,41	0.20	0,10
G1-L	1,92	0.16	2,90

365 * Information provided by the product labeling.

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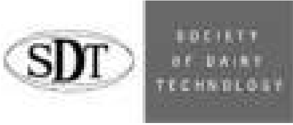
PUBLICACIÓN 8

Conjugated linoleic acid content in fermented goat milk as affected by the starter culture and the presence of free linoleic acid

Lorena Trigueros, Xavier Barber, Esther Sendra

International Journal of Dairy Technology



CONJUGATED LINOLEIC ACID CONTENT IN FERMENTED GOAT MILK AS AFFECTED BY THE STARTER CULTURE AND THE PRESENCE OF FREE LINOLEIC ACID

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CONJUGATED LINOLEIC ACID CONTENT IN FERMENTED GOAT MILK AS AFFECTED BY THE STARTER CULTURE AND THE PRESENCE OF FREE LINOLEIC ACID

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CLA CONTENT IN FERMENTED GOAT MILK

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Abstract: The effect of yogurt starter cultures and probiotic *L. casei* on the formation of conjugated linoleic acid (CLA) and microbial populations of fermented goat milk was investigated during 35 days of cold storage. The addition of hydrolyzed sunflower oil (HSO) as a source of free linoleic acid was investigated. Fermentation process enhanced the content of *cis*9, *trans*11-CLA isomer in milk, whereas the *trans*10, *cis*12-CLA isomer was not detected in goat milk or control fermented milks. The use of both starters generated *trans*10, *cis*12-CLA only when HSO was supplemented. Populations of streptococci and lactobacilli were affected by the presence of HSO.

KEYWORDS: goat milk, conjugated linoleic acid, hydrolyzed sunflower oil, Tonalin®, probiotic

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49 **INTRODUCTION**

50 Conjugated linoleic acid (CLA) is the collective name given to a group of linoleic acid
51 (C18, *cis*9: *cis*12) free fatty acid positional and geometric isomers, containing two
52 conjugated double bonds (Fernie 2003). Of the individual isomers of CLA, *cis*-9, *trans*-
53 11-octadecadienoic acid (*cis*9, *trans*11-CLA) and *trans*-10, *cis*-12-octadecadienoic acid
54 (*trans*10, *cis*12-CLA) are the main isomers found in the diet and also have been
55 implicated as the most biologically active (Li *et al*. 2012). CLA isomers have attracted
56 considerable interest up to date because of their potentially beneficial biologic effects
57 of attenuating lifestyle-related diseases; the *trans*10, *cis*12-CLA isomer has
58 demonstrated anticarcinogenic, antiobese and antidiabetic effects whereas the *cis*9,
59 *trans*11-CLA isomer exerts an anticancer effect (Trigueros *et al*. 2013).

60 Dairy products are the most important source of CLA from animal foodstuffs, which
61 content ranges from 3.59 to 7.96 mg/g fat (Lin *et al*. 1995). Variability in the CLA
62 content of cheeses, yogurts, and other commercial dairy products depends on the CLA
63 content of the raw milk, starter cultures, aging time, and other processing treatments
64 (Xu *et al*. 2004). Goat milk has higher CLA content (7 mg/ g fat) than cow milk (6 mg/ g
65 fat), as the sum of total CLAs (Tamime *et al*. 2011).

66 A number of studies have focused on the ability of probiotic bacteria to form CLA in
67 model systems with linoleic acid. In the study carried out by Lin *et al*. (1999) six lactic
68 cultures of lactobacilli, lactococci and streptococci were able to produce CLA *in vitro* in
69 sterilized skim milk containing free linoleic acid;. Two strains of *Lactobacillus*
70 *acidophilus* and *L. casei* proved their ability to produce CLA from linoleic acid in MRS
71 broth and in skim milk supplemented with linoleic acid (Alonso *et al*. 2003). Xu *et al*.
72 (2004) demonstrated that the CLA-producing ability of eleven lactic acid and probiotic

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bacteria in a milk model system depends on the strain of probiotic bacteria but also on the lipid source. All tested strains were capable to produce CLA but only from the hydrolyzed oil.

Goat milk is an excellent raw material as well as a valuable food product. The profile of fatty acids is more beneficial in goat milk compared to sheep and bovine milk because of the unsaturated fatty acids/saturated fatty acids (UFA/SFA) ratio 0.56, of 0.52 (sheep milk) vs. 0.37 (bovine milk) (Bernacka 2011). Goat milk exhibits health attributes that could be advantageous for specific population groups such as infants, athletes and the elderly (Michaelidou 2008). The variety of studies of fermented milk products from goat milk includes set-type fermented milk (Martín-Diana *et al*. 2003), concentrated yogurt (Malek *et al*. 2001), fruit yogurt (Senaka Ranadheera *et al*. 2012) and probiotic yogurt (Kudelka 2010).

Enhancing CLA in dietary components is a potential way of increasing CLA intake. Fermented milk products are good candidates as vehicles for CLA due to the ability of lactic acid bacteria to produce CLA from linoleic acid (Jiang *et al*. 1998). However, the CLA production in goat milk fermented by lactic acid bacteria has not been reported. The objective of the present work was to determine the ability of common yogurt starter cultures (*L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and widely commercially used probiotic *L. casei* to produce CLA in whole goat milk supplemented with a linoleic acid source. CLA content and microbial counts were determined during 35-days of refrigerated storage period of fermented goat's milk.

MATERIALS AND METHODS

MATERIALS

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1
2
3 96 Raw whole goat milk was collected from the Miguel Hernandez University farm
4
5 97 (Alicante, Spain). Milk was collected about 24 hours after milking; the temperature of
6
7
8 98 the milk tank was of 3°C and the pH 6.6. Three independent batches of raw milk were
9
10 99 collected during March 2012. Each sampling day 6 liters of milk were used (1liter for each
11
12 100 treatment: 2 types of culture*3 types of fermented milk). Hydrolyzed sunflower oil (HSO)
13
14
15 101 was prepared from sunflower oil (Hacendado, Sevilla, Spain) according to the method
16
17 102 described by Xu *et al*. (2004). The HSO was dissolved in Tween-80 solution
18
19 103 (polyoxyethylene sorbitan mono-oleate) (tween 80: water = 1:99, w/w) at a 1:1 (w/w)
20
21
22 104 ratio to form a stable emulsion suitable for incorporation of the oil into the milk.
23
24 105 All reagents used in the lab procedure were GC grade. Fatty acid methyl esters (FAME)
25
26 106 Mix Supelco GLC-50, GLC-100, CLA isomers (C18:2 *cis*9, *trans*11 and C18:2 *trans*10,
27
28
29 107 *cis*12), nonanoic acid (C9:0) and heptadecanoic acid (C17:0) and n-hexadecane (cetane)
30
31 108 were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and high CLA
32
33 109 content powder (Tonalin® 60 WDP) was supplied by BASF Personal Care and Nutrition
34
35
36 110 (GmbH, Germany).
37
38 111 BACTERIAL CULTURES
39
40
41 112 Traditional yogurt culture (1:1 ratio of *L. delbrueckii* subsp. *bulgaricus* and *S.*
42
43 113 *thermophilus*, YF-L811 Yo-Flex®) and *L. casei* (LC-01 nu-trish®) was supplied by Chr.
44
45 114 Hansen (Hørsholm, Denmark) and used at the concentrations recommended by the
46
47
48 115 suppliers. The cultures were subcultured al least 3 times in 10% skim milk immediately
49
50 116 before experimentation using 1% inocula and an 18-h incubation at 37°C.
51
52 117 CHEMICAL ANALYSIS
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118 The chemical composition of goat milk was automatically determined in a Milko Scan
119 FT 120 (Foss Elctric, Hillerød, Denmark). The pH was measured using a pH-meter
120 (model pH/Ion 510, Eutech Instruments Pte Ltd., Singapore).

121 FERMENTED MILKS MANUFACTURE

122 Raw goat milk was poured into 1-L Pyrex© flasks, closed and immersed into a water
123 bath for heat treatment at 80°C for 30 min. Control samples, each of yogurt cultures
124 (Y) or *L. casei* (Lc), were prepared. The emulsion prepared from HSO (1% in final
125 products) was supplemented to each fermented milk group. In order to confirm the
126 results, a group containing 0.5% (in final products) of Tonalin® (80% CLA) was
127 prepared. Each mixture was pasteurized at 80°C for 30 min, cooled to 43°C, and
128 inoculated at 2% with *L. casei* or yogurt cultures previously activated . The inoculated
129 mixes were incubated at 37°C for *L. casei* or 43°C for yogurt cultures until a pH of 3.7
130 (*L. casei*) or 4.7 (yogurts) and then cooled down to 4°C. Then the samples were stored
131 for 35 days at 4°C. Samples were analyzed at the end-point of the fermentation (0),
132 and at 1, 14, 28 and 35 days during storage.

133 LIPID ANALYSIS

134 *Reaction products from hydrolysis process*

135 Reaction products from the hydrolysis of sunflower oil were monitored by capillary
136 column gas chromatography (Luna *et al*. 2013), using a Hewlett-Packard 5890 series II
137 equipped with a flame ionization detector (FID) and a HT5 capillary column (25 m x
138 0,32 mm x 0,10 µmSGE, Supelco) with a flame ionization detector (FID) at 450°C and
139 splitless injection at 350°C. As carrier gas helium is used, with a flow of 1.5 ml/min, it
140 has been applied a heating ramp from 45°C to 200°C at a rate of 7°C/min, followed by
141 another ramp from 200$_{o}$C to 360°C at a rate of 15oC/min, maintaining the oven

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142 temperature at 360°C for 10 min, using as internal standard n-hexadecane (cetane) to
143 quantify the content of methyl esters and glycerides (-mono, di and triglycerides) with
144 the help of some commercial standard fatty acid esters, respectively (Calero *et al.*
145 2014).

146 Considering that sunflower oil is constituted by a mixture of fatty acids in variable
147 proportion (mainly linoleic, oleic and stearic acids), reactions results are expressed as
148 the relative amounts of the corresponding methyl esters (FAME), monoglycerides (MG)
149 and diglycerides (DG) that are integrated in the chromatogram. By difference and
150 respect to the internal standard (cetane), the amount of triglycerides (TG) which has
151 not reacted, is calculated.

152 *Methylation procedure*

153 Fatty acids were *in situ* methylated according to the Park and Goins (1994) method
154 with some modifications. Basically, 50 mg of HSO, 150 µL of goat milk or 250 µL of
155 fermented milk were transferred into a test tube with 40 µL of C9:0 and 80 µL of C17:0
156 *n*-hexane solutions (20 mg/mL) as internal standards. Then, 100 µL of methylene
157 chloride and 1 mL of 0.5N NaOH in methanol were added and the tubes were heated
158 in a water bath at 90°C for 10 min. One mL of BF_3 in methanol was added and the
159 mixture was left at room temperature (25°C) for 30 min to prevent intraisomerization
160 of CLA isomers. One mL of distilled water and 600 µL hexane were added and then
161 FAMEs were extracted by vigorous shaking for about 1 min. Following centrifugation,
162 the aliquots were dried with anhydrus sodium sulphate and the top layer was
163 transferred into a vial flushed with nitrogen which was stored at -20°C until analyzed
164 by gas chromatography.

165 *Gas chromatography (GC) analysis*

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The fatty acid composition of FAMEs was analyzed on a Agilent gas chromatography unit (model 6890, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a DB-23 capillary column (30 m length, 0.25 µm film, 0.25 mm internal diameter; J&W Scientific, Agilent Technologies). The flow rate of the carrier gas (Helium) was 1.2 mL/min and 35 mL/min at the make-up point, the injector temperature was 200°C and the detector 220°C. The injection volume was 0.5 µL (splitless). The temperature program was as follows: initial temperature 70°C, temperature from 70 to 190°C at 10°C/min for 20 min, from 190 to 240°C at 5°C/min and at 240°C held isothermally for 3 min.

Identification of the FAME peaks was performed by comparing the retention times of the FAME standards. Agilent technologies software (G2072AA Rev.A.05.02 Chemstation) was used for integration of peaks. CLA and fatty acid concentrations in fermented milks were expressed as percentage of total fatty acids using response factors which were previously determined.

MICROBIOLOGICAL ANALYSIS

Lactobacillus spp. was enumerated on MRS agar (Cultimed, Panreac, Castellar del Vallés, Barcelona, Spain) under anaerobic incubation at 37°C for 48 h. *Streptococcus* spp. were counted using M-17 agar (Scharlau, Barcelona, Spain) incubated aerobically at 37°C for 24 h.

STATISTICAL ANALYSIS

We used mixed models for repeated measures (Pinheiro and Bates 2000; Venables and Ripley 2002), as a fixed effects the lipid source (control, HSO, Tonalin®) culture type (yogurt, *L. casei*) and as a mixed effects the storage time effect (0, 1, 14, 28, 35) on fermented milk samples. The model was adjusted using R software (R Core Team 2013,

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190 URL http://www.R-project.org/). The mean differences after analysis were evaluated
191 using Tukey's HSD post-hoc test. The whole experiment was independently run three
192 times.

193 **RESULTS AND DISCUSSION**

194 GOAT MILK COMPOSITION

195 The chemical composition of the raw goat milk was: 6.38% fat, 4.31% protein, 3.41%
196 casein, 4.27% lactose, 15.36% dry matter, 0.69% (expressed in g of oleic acid in 100 g
197 fat) free fatty acids and pH of 6.60. We found higher fat content than the reported by
198 the literature (Boyazoglu and Morand-Fehr 2001; Tamime *et al*. 2011), however the
199 chemical composition of any type of fresh milk varies over time depending on such
200 factors as breed, individual animal, diet and feeding, environmental and regional
201 (local) conditions, lactation period, health status, etc. (Slačanac *et al*. 2010).

202 FERMENTATION PROCESS

203 The presence of HSO or Tonalin® in goat milk did not affect the fermentation process
204 (*p-value* > 0.05) neither in yogurt nor *L. casei* fermented milks (data not shown). After
205 incubation the pH of control yogurt (CY), hydrolyzed sunflower oil yogurt (HY) and
206 Tonalin® yogurt (TY) was 4.69, 4.67 and 4.69 respectively. For *L. casei* fermented milks
207 final pH reached was 3.63 for control (CLc), 3.54 for hydrolyzed sunflower oil (HLc) and
208 3.56 for Tonalin® (TLc).

209 The effect of the different lactic acid cultures on the fatty acid profile of goat milk is
210 presented in Table 1. The addition of yogurt cultures and *L.casei*, although slightly,
211 significantly increased the content of *cis*9, *trans*11-CLA. As goat milk was not
212 supplemented in that case this increase of the *cis*9, *trans*11-isomer of CLA was
213 probably due to fermentation. No major changes were observed in C10:0, C14:0,

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214 C16:0, C18:0 and C18:1 which are goat milk's major fatty acids. Furthermore, no
215 *trans*10, *cis*12-CLA isomer was detected in the non-supplemented samples.

216 pH EVOLUTION DURING STORAGE TIME

217 *Yogurt*

218 Over all the storage period no differences in pH were detected between yogurts.
219 Average pH values for CY, HY and TY were 4.27±0.08, 4.17±0.15 and 4.21±0.25,
220 respectively. pH values of yogurts were higher at 0 and 1 day of storage (*p-value* <
221 0.05) than the rest of the days. pH value of TY decreased gradually during the storage
222 time; pH of CY and HY slightly increased 28th day and 35th day but this was statistically
223 not significant (*p-value* = 0.542).

224 *L. casei fermented goat milk*

225 Like yogurts, *L. casei* fermented milks presented similar pH values over the storage
226 period being not statistically significant (average pH values for CLc, HLc and TLc were
227 3.42±0.04, 3.40±0.05 and 3.43±0.09, respectively). In general, pH decreased
228 throughout the storage period. During the first 24 hours pH was higher (*p-value* < 0.05)
229 than that on 14th day and 28th day presenting initial pH values of 3.47, 3.46 and 3.56
230 for CLc, HLc and TLc; however on 35th day, although slightly, pH increased significantly
231 reaching pH values of 3.40, 3.40 and 3.38 for CLc, HLc and TLc.

232 FATTY ACIDS EVOLUTION OF FERMENTED MILKS DURING STORAGE

233 *Preliminary assays*

234 Preliminary assays with unhydrolyzed sunflower oil (SO) were conducted (data not
235 shown). The content of linoleic acid remained unchanged at the end point of
236 fermentation and during the storage period both in yogurt and *L. casei* fermented
237 milks. Also, the CLA content remained unaltered. So, the lactic acid bacteria used in

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238 this study were unable to use the oil when is presented in the unhydrolyzed form to
239 produce CLA. These findings are in agreement with those of Xu *et al*. (2004) and
240 Gorissen *et al*. (2012). To the best understanding of the bacterial cultures behavior the
241 ratio of hydrolysis of SO and HSO was calculated. As can be observed in Table 2. the
242 HSO used in the present study was completely hydrolyzed.

243 *Yogurt*

244 We focused the study on the fatty acids presented in Table 3. The supplementation
245 with HSO or Tonalin® dramatically affected the fatty acid profile of the yogurt. As the
246 HSO used in this experiment contained 61.68% of linoleic acid (in total fatty acids) the
247 content of such fatty acid was statistically higher in HY. For the same reason content of
248 CLA isomers were higher (*p-value* < 0.05) in TY (Tonalin® consists in 80% total CLA). TY
249 was manufactured in order to serve as reference CLA enriched samples. The increase
250 in CLA levels due to yogurt fermentation has been already evidenced in Table 1. In the
251 present study, HSO (as a substrate for linoleic acid) was supplemented to goat's milk to
252 evaluate possible acceleration in the production of CLA, which was also reported in
253 prior studies (Colakoglu and Gursoy 2011; Xu *et al*. 2005). Colakoglu *et al*. (2011)
254 showed that the CLA concentration of a yogurt supplemented with sunflower oil
255 (0.1%) and *L. reuteri* ATCC 55739 as added adjunct culture increased by 2.3%. Xu *et al*.
256 (2005) demonstrated that the combination of probiotic bacteria with yogurt culture
257 did not produced significantly higher amounts of CLA than yogurt culture alone when
258 HSO was used as substrate. In our study, we found that yogurt starter culture did not
259 increase the concentration of the *cis*9, *trans*11-CLA isomer, but was able to produce
260 the *trans*10, *cis*12-CLA isomer when using free linoleic acid as a source. The high free
261 fatty acid content of goat milk (0.69% in total fat) together with the high level of

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262 linoleic acid supplemented may have acted as enhancers or initiators of the
263 isomerisation of CLA (Gorissen *et al*. 2012). Maximum CLA production in HY (1.02% of
264 total fatty acids for the *cis*9, *trans*11-CLA isomer and 0.18% for the *trans*10, *cis*12-CLA
265 isomer) was achieved at the 28th day of cold storage. The review carried out by Sieber
266 *et al*. (2004) pointed out that strains of lactobacilli, bifidobacteria and propionibacteria
267 were able to successfully convert linoleic acid to CLA although in some cases did not
268 produce it at high levels, especially when dairy products as yogurt or cheese are
269 manufactured, may be due to the fact that they were not elaborated with specific CLA-
270 producing lactic acid bacteria strains. However, Gorissen *et al*. (2012) did not observe
271 an increase in the CLA concentration when milk was fermented with different
272 bifidobacteria strains and yogurt cultures, not even in milk supplemented with
273 vegetable oils (non hydrolyzed). In general, no differences were found in the fatty acid
274 content during the storage period (*p-value* > 0.05).

275 *L. casei fermented goat milk*

276 *L casei* proved to increase CLA content as seen in Table 1. As occured in yogurt, the
277 content of linoleic acid was statistically higher in HLc (Table 4). The content of CLA
278 isomers were higher (*p-value* < 0.05) in TLc. Maximum production of the *cis*9, *trans*11-
279 CLA isomer in HLc was observed at the 14th day (0.99%) and at 28th day for the
280 *trans*10, *cis*12 CLA-isomer (0.17%). Whereas the level of the *cis*9, *trans*11-CLA isomer
281 remained stable during the storage period, the content of the *trans*10, *cis*12-CLA
282 isomer was gradually increased during storage at 4°C both in HLc and TLc (*p-value* >
283 0.05) until the 28th day. Li *et al*. (2012) proved that the effect of the incorporation of
284 probiotic bacteria in enhancing CLA content is dependent on the specific probiotic
285 bacteria strain. They also reported that during 14 days of storage the CLA contents

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significantly decreased. Other authors studied different strains of probiotic bacteria for its ability to convert linoleic acid to CLA from free linoleic acid in fermented milk products (Alonso *et al*. 2003; Lin 2003). Puniya *et al*. (2009) stated that *L. casei* could be a potential CLA producer demonstrating *in vitro* its ability to produce higher CLA level at higher unhydrolyzed sunflower oil concentration.

MICROBIOLOGY OF FERMENTED MILKS DURING STORAGE

Yogurt

The use of different lipid sources, storage time and their interaction had a significant influence on *S. thermophilus* counts and *Lactobacillus* spp. counts (*p-value* < 0.01) (Figure 1). At the 28th day of cold storage numbers of *S. thermophilus* and *Lactobacillus* spp. decreased significantly when HSO was used as lipid source. The antibacterial effect of free linoleic acid has been known for many years. The inhibitory effect is dependent on the bacterial strains and the levels and availability of the fatty acid. Xu *et al*. (2004) did not observe such inhibitory effect which was attributed to the use of an acacia as emulsifier which may have prevented the inhibitory effect. We used Tween-80 as emulsifier for the hydrolyzed oil, the high concentration (1%) of hydrolyzed oil could have affected yogurt culture population. In general, *S. thermophilus* counts of yogurts had a tendency to decrease during the storage period. Regarding *Lactobacillus* spp., their counts decreased to the 14th day of storage and then increased until the end of storage in CY and TY. Counts of *Lactobacillus* spp. in HY were almost unchanged during cold storage.

L. casei fermented goat milk

No two-way interaction effects were found for lipid source and storage time on *L. casei* growth. Changes in *L. casei* counts during storage were found statistically significant

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310 (p-*value* < 0.05) (Figure 2). While *L. casei* counts were similar at zero, 1st and 14th days
311 of storage, counts were lower at day 28th and 35th (p-*value* < 0.05).
312

313 **CONCLUSIONS**

314 The fermentation process positively affects the content of *cis*9, *trans*11-CLA isomer,
315 whereas the *trans*10, *cis*12-CLA isomer is not detected in goat milk or fermented goat
316 milk. The ability to produce *trans*10, *cis*12-CLA by starter bacteria depends on the
317 presence of free linoleic acid (HSO). The starter cultures tested as well as the storage
318 time evaluated do not affect CLA contents in fermented goat milk. The presence of
319 HSO inhibits the growth of streptococci and lactobacilli, although their counts
320 remained over the minimum requirements of viable cells at the time of consumption
321 by Spanish legislation (10^7) (R.D. 179/2003, B.O.E. 18/02/03). Both yogurt culture and
322 *L. casei* produce *trans*10, *cis*12-CLA only when HSO is used as lipid source. Future
323 studies are needed to reduce the concentration of HSO or find an alternative source of
324 free linoleic acid.
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326 **REFERENCES**

327 Alonso L, Cuesta E P and Gilliland E (2003) Production of free conjugated linoleic acid
328 by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin.
329 *Journal of Dairy Science* **86** 1941-1946.
330 Bernacka H (2011) Health-promoting properties of goat milk. *Medycyna Weterynaryjna*
331 **67** 507-511.
332 Boyazoglu J and Morand-Fehr (2001) Mediterranean dairy sheep and goat products
333 and their quality. A critical review. *Small Ruminant Research* **40** 1-11.

For Peer Review

1
2
3 334 Calero J, Luna D, Sancho E D, Luna C, Bautista F M, Romero A A, Posadillo A and
4
5 335 Verdugo C (2014) Development of a new biodiesel that integrates glycerol, by
6
7
8 336 using CaO as heterogeneous catalyst, in the partial methanolysis of sunflower
9
10 337 oil. *Fuel* **122** 94-102.
11
12 338 Colakoglu H and Gursoy O (2011) Effect of lactic adjunct cultures on conjugated linoleic
13
14
15 339 acid (CLA) concentration of yogurt drink. *Journal of Food, Agriculture &*
16
17 340 *Environment* **9** 60-64.
18
19 341 Fernie C E (2003) Conjugated linoleic acid. In *Encyclopaedia of Food Science and*
20
21
22 342 *Nutrition* (2nd ed.), pp. 1581-1587. Caballero B, ed. London: Elsevier Science.
23
24 343 Gorissen L, Raes K, De Smet S, De Vuyst L and Leroy F (2012) Microbial production of
25
26
27 344 conjugated linoleic and linolenic acids in fermented foods: technological
28
29 345 bottlenecks. *European Journal of Lipid Science and Technology* **114** 486-491.
30
31 346 Jiang J, Björck L and Fondén R (1998) Production of conjugated linoleic acid by dairy
32
33
34 347 starter cultures. *Journal of Applied Microbiology* **85** 95-102.
35
36 348 Kudelka W (2010) Probiotics in natural bio-yoghurts of goat's milk. *Milchwissenschaft*
37
38
39 349 **65** 407-410.
40
41 350 Li H, Liu Y, Bao Y, Liu X and Zhang H (2012) Conjugated linoleic acid conversion by six
42
43 351 *Lactobacillus plantarum* strains cultured in MRS broth supplemented with
44
45
46 352 sunflower oil and soymilk. *Journal of Food Science* **77** M330-M336.
47
48 353 Lin H, Boylston T D, Chang M J, Luedecke L O and Shultz T D (1995) Survey of the
49
50 354 conjugated linoleic acid contents of dairy products. *Journal of Dairy Science* **78**
51
52
53 355 2358-2365.
54
55 356 Lin T Y, Lin C-W and Lee C-H (1999) Conjugated linoleic acid concentration as affected
56
57 357 by lactic cultures and added linoleic acid. *Food Chemistry* **67** 1-5.
58
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For Peer Review

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358 Lin T Y (2003) Influence of lactic cultures, linoleic acid and fructo-oligosaccharides on
359 conjugated linoleic acid concentration in non-fat set yogurt. *Australian Journal*
360 *of Dairy Technology* **58** 11-14.
361 Luna C, Sancho E, Luna D, Caballero V, Calero J, Posadillo A, Verdugo C, Bautista F M,
362 and Romero A A (2013) Biofuel that keeps glycerol as monoglyceride by 1,3-
363 selective ethanolysis with pig pancreatic lipase covalently immobilized on $AIPO_4$
364 support. *Energies* **6** 3879-3900.
365 Malek A, Shadarevian S and Toufeili I (2001) Sensory properties and consumer
366 acceptance of concentrated yogurt made from cow's, goat's and sheep's milk.
367 *Milchwissenschaft* **56** 687-690.
368 Martín-Diana A B, Janer C, Peláez C and Requena T (2003) Development of a fermented
369 goat's milk containing probiotic bacteria. *International Dairy Journal* **13** 827-
370 833.
371 Michaelidou A M (2008) Factors influencing nutritional and health profile of milk and
372 milk products. *Small Ruminant Research* **79** 42-50.
373 Park P W and Goins R E (1994) *In situ* preparation of fatty acid methyl esters for
374 analysis of fatty acid composition in foods. *Journal of Food Science* **59** 1262-
375 1266.
376 Pinheiro J C and Bates D M (2000) *Mixed effects models in S and S-PLUS*. New York:
377 Springer-Verlag.
378 Puniya A K, Reddy C S, Kumar S and Singh K (2009) Influence of sunflower oil on
379 conjugated linoleic acid production by *Lactobacillus acidophilus* and
380 *Lactobacillus casei*. *Annals of Microbiology* **59** 505-507.

For Peer Review

Senaka Ranadheera C, Evans C A, Adams M C and Baines S K (2012) Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chemistry* **135** 1411-1418.

Sieber R, Collomb M, Aeschlimann A, Jelen P and Eyer H (2004) Impact of microbial cultures on conjugated linoleic acid in dairy products - a review. *International Dairy Journal* **14** 1-15.

Slačanac V, Božanić R, Hardi J, Szabó J R, Lučan M and Krstanović V (2010) Nutritional and therapeutic value of fermented caprine milk. *International Journal of Dairy Technology* **63** 171-189.

Tamime A Y, Wszolek M, Božanić R and Özer B (2011) Popular ovine and caprine fermented milks. *Small Ruminant Research* **101** 2-16.

Trigueros L, Peña S, Ugidos A V, Sayas-Barberá E, Pérez-Álvarez J A and Sendra E (2013) Food ingredients as anti-obesity agents: a review. *Critical Reviews in Food Science and Nutrition* **53** 929-942.

Xu S, Boylston T D and Glatz B A (2004) Effect of lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. *Journal of the American Oil Chemists' Society* **81** 589-595.

Xu S, Boylston T D and Glatz B A (2005) Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. *Journal of Agricultural and Food Chemistry* **3** 9064-9072.

For Peer Review

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405 Figure 1. The effect of storage time on *S. thermophilus* (St) counts estimated in M17
406 agar and *Lactobacillus* spp. (Lb) counts estimated in MRS agar of goat's yogurts with
407 different lipid sources [control (C), yogurt with hydrolyzed sunflower oil (H) and yogurt
408 with Tonalin® (T)].

409

410 Figure 2. The effect of storage time on *L. casei* counts estimated in MRS agar of goat's
411 *L. casei* fermented milk with different lipid sources [control (C), yogurt with hydrolyzed
412 sunflower oil (H) and yogurt with Tonalin® (T)].

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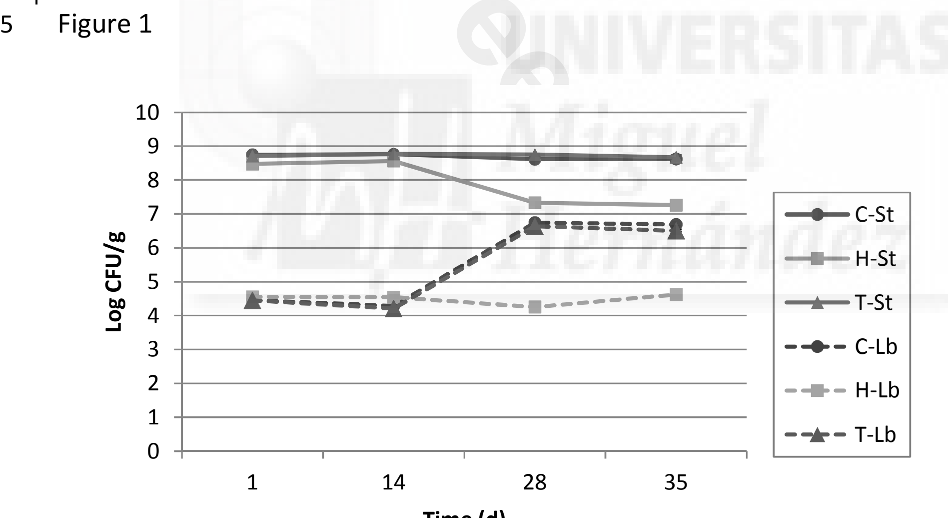
415 Figure 1



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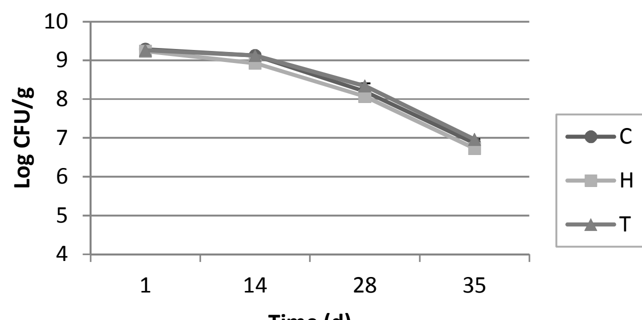
422 Figure 2



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426 **Tables**

427 Table 1. Average values for fatty acid profile (% of total fatty acids) of raw milk and

428 control fermented goat milks (yogurt and *L casei*) (24 hours after manufacture)

	Raw goat milk		Yogurt		*L. casei*	
Fatty acid	Mean	SD	Mean	SD	Mean	SD
C4:0	0.69	0.09	0.56	0.13	0.68	0.05
C6:0	1.74	0.24	1.52	0.07	1.82	0.32
C8:0	2.56	0.46	2.26	0.13	2.71	0.57
C10:0	8.46	1.12	7.64	0.32	8.70	1.98
C12:0	3.78	0.16	3.64	0.19	3.98	0.68
C14:0	9.11	0.33	8.85	0.70	8.83	0.79
C15:0	0.78	0.22	0.74	0.19	0.76	0.19
C16:0	26.61	0.37	27.09	0.20	26.31	0.90
C16:1	1.22	0.14	1.20	0.14	0.93	0.63
C18:0	13.27	0.12	14.03	0.93	13.36	0.56
C18:1	26.93	1.01	27.62	0.54	27.01	1.22
C18:2	2.79	0.15	2.86	0.24	2.74	0.13
C18:3	0.08	0.04	0.08	0.03	0.16	0.12
C18:2 *c*9,*t*11-CLA	0.81^{a}	0.07	0.99^{b}	0.04	1.00^{b}	0.14
C18:2 *t*10,*c*12-CLA	nd	---	nd	---	nd	---
C20:0	0.25	0.09	0.27	0.05	0.28	0.08
C20:1	0.74	0.08	0.33	0.20	0.58	0.05
SFA	67.26	0.96	66.60	0.59	67.45	1.90
MUFA	28.89	1.07	29.15	0.92	28.52	1.71
PUFA	3.68	0.18	3.93	0.27	3.90	0.28
ΣC18	43.88	1.00	45.58	1.00	44.27	1.83
ΣCLA	0.81	0.03	0.99	0.02	1.00	0.07

429 Values within the same row followed by different superscript letters significantly differ (*p-value* < 0.05).
430 Abbreviation: not detected (nd), standard deviation (SD), *trans* (*t*), *cis* (*c*), conjugated linoleic acid (CLA),
431 saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).
432 SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C20:0; MUFA: sum of C16:1,
433 C18:1 and C20:1; PUFA: sum of all C18:2 and C18:3.
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3 435 Table 2. Percentage of hydrolysis of the sunflower oil.

%	HSO (%)	SO
FFA	60.08	0
MG	19.12	4.72
DG	19.96	9.88
GLY	0.84	0
TG	0	85.4

436 Abbreviation: HSO, hydrolyzed sunflower oil; SO, sunflower oil; FFA, free fatty acids; MG,
437 monoglycerides; DG, diglycerides, GLY, glycerine, TG, triglycerides.
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3 439 Table 3a. Content of C18's and major groups of fatty acids (% of total fatty acids) in
4
5 440 goat's milk yogurt as a function of lipid source and cold storage time
6

	Time (days)				
	0	1	14	28	35
C18:2					
Control	2.96±0.33	2.88±0.05	2.73±0.01	2.71±0.04	2.72±0.11
Hydrolyzed	10.96±0.97	10.34±0.12	9.50±0.21	9.21±0.14	11.25±0.03
Tonalin®	2.76±0.34	2.67±0.02	2.65±0.05	2.73±0.05	2.52±0.01
C18:2 *c*9,*t*11-CLA					
Control	0.97±0.02	0.98±0.04	0.96±0.01	0.93±0.03	0.93±0.02
Hydrolyzed	0.96±0.05	1.00±0.06	0.94±0.03	1.02±0.01	0.93±0.03
Tonalin®	1.39±0.27	1.32±0.11	1.27±0.04	1.27±0.04	1.31±0.09
C18:2 *t*10,*c*12-CLA					
Control	nd	nd	nd	nd	nd
Hydrolyzed	0.14±0.01	0.08±0.03	0.11±0.05	0.18±0.05	0.15±0.01
Tonalin®	0.24±0.04	0.23±0.06	0.12±0.02	0.13±0.03	0.17±0.04
ΣC18					
Control	46.25±1.07	44.24±0.66	45.08±0.37	45.27±0.13	44.15±0.59
Hydrolyzed	53.00±1.01	52.23±9.59	51.43±0.88	52.81±0.39	52.06±2.37
Tonalin®	47.10±4.64	45.14±0.26	47.58±0.97	47.19±0.31	46.07±1.02
SFA					
Control	67.01±0.63	66.57±0.45	67.67±0.16	67.70±0.05	69.09±1.23
Hydrolyzed	57.35±1.46	58.11±11.59	59.65±0.39	58.70±0.60	57.93±1.53
Tonalin®	64.02±3.55	65.25±0.32	64.57±0.44	64.88±0.06	66.00±0.76
MUFA					
Control	28.45±0.77	28.66±0.69	28.22±0.17	28.23±0.10	27.81±0.11
Hydrolyzed	30.24±0.37	29.73±1.90	29.39±0.73	30.42±0.14	29.35±1.51
Tonalin®	29.61±2.72	29.83±0.02	30.38±0.40	30.15±0.14	29.22±0.59
PUFA					
Control	4.10±0.30	4.06±0.06	3.86±0.06	3.79±0.13	3.80±0.16
Hydrolyzed	12.23±1.04	11.63±0.17	10.67±0.19	10.56±0.21	12.48±0.01
Tonalin®	5.37±0.79	4.35±0.33	4.23±0.04	4.31±0.04	4.20±0.15

441 Presented values are means of triplicate determinations.
442 ± indicates standard deviation from the mean.
443 Abbreviation: not detected (nd), *trans* (*t*), *cis* (*c*), conjugated linoleic acid (CLA), saturated fatty acids
444 (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).
445 SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C20:0; MUFA: sum of C16:1,
446 C18:1 and C20:1; PUFA: sum of all C18:2 and C18:3.
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452 Table 3b. Homoegenous groups between means as determined by Tukey's HSD Test (*p*-
453 *value* < 0.05) for C18's and major groups of fatty acids (% of total fatty acids) in goat's
454 milk yogurt as a function of lipid source and cold storage time.

Factors	Lipid source			Time (days)				
	Control	Hydrolyzed	Tonalin®	0	1	14	28	35
C18:2	a	b	a	a	a	a	a	a
C18:2 *c*-9,*t*-11 CLA	a	a	b	a	a	a	a	a
C18:2 *t*-10,*c*-12 CLA	a	b	b	a	a	a	a	a
ΣC18	a	b	a	a	a	a	a	a
SFA	b	a	b	a	a	a	a	a
MUFA	a	b	b	a	a	a	a	a
PUFA	a	b	a	a	a	a	a	a

455 Abbreviation: *trans* (*t*), *cis* (*c*), conjugated linoleic acid (CLA), saturated fatty acids (SFA),
456 monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).
457 SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C20:0; MUFA: sum of C16:1,
458 C18:1 and C20:1; PUFA: sum of all C18:2 and C18:3.
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473 Table 4a. C18's and major groups of fatty acids (% of total fatty acids) in *L. casei*

474 fermented goat's milk as a function of lipid source and cold storage time

	Time (days)				
	0	1	14	28	35
C18:2					
Control	2,79±0,06	2,83±0,26	2,75±0,05	2,70±0,02	2,69±0,09
Hydrolyzed	10,49±0,58	11,14±0,42	9,23±0,23	9,81±0,36	10,81±0,03
Tonalin®	2,61±0,15	2,68±0,05	2,74±0,02	2,66±0,05	2,58±0,14
C18:2 *c*9,*t*11-CLA					
Control	0,90±0,07	0,98±0,01	0,91±0,03	0,94±0,01	0,94±0.00
Hydrolyzed	0,89±0,01	0,95±0,01	0,99±0,08	0,96±0,06	0,88±0,02
Tonalin®	1,32±0,21	1,25±0,14	1,31±0,04	1,40±0,00	1,35±0,36
C18:2 *t*10,*c*12-CLA					
Control	nd	nd	nd	nd	nd
Hydrolyzed	0,10±0,00	0,15±0,04	0,15±0,02	0,17±0,02	0,12±0,01
Tonalin®	0,16±0,02	0,17±0,00	0,23±0,01	0,28±0,04	0,27±0,03
ΣC18					
Control	43,28±0,45	44,14±0,27	45,65±0,64	45,03±0,02	44,28±0,14
Hydrolyzed	51,78±0,30	53,17±0,08	52,34±0,37	52,34±0,31	50,03±0,39
Tonalin®	46,02±0,28	45,05±1,60	49,09±0,54	46,80±0,50	46,59±1,21
SFA					
Control	68,75±0,95	67,93±1,00	67,49±0,20	67,83±0,06	68,42±0,02
Hydrolyzed	58,38±0,33	56,42±0,11	58,59±0,95	58,71±0,29	59,79±0,33
Tonalin®	67,46±0,47	65,99±1,09	67,07±0,33	65,16±0,96	65,74±0,89
MUFA					
Control	27,26±0,98	27,86±0,85	28,51±0,21	28,04±0,02	27,70±0,06
Hydrolyzed	29,84±0,28	30,85±0,83	30,59±0,53	29,95±0,23	28,08±0,42
Tonalin®	29,96±0,52	29,55±0,77	31,21±0,47	29,74±0,62	29,04±0,30
PUFA					
Control	3,90±0,04	4,00±0,24	3,79±0,11	3,82±0,01	3,77±0,08
Hydrolyzed	11,64±0,63	12,38±0,51	10,52±0,33	11,08±0,31	11,92±0,05
Tonalin®	4,22±0,15	4,21±0,35	4,47±0,06	4,53±0,06	4,92±0,55

475 Presented values are means of triplicate determinations.
476 ± indicates standard deviation from the mean.
477 Abbreviation: not detected (nd), *trans* (*t*), *cis* (*c*), conjugated linoleic acid (CLA), saturated fatty acids
478 (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).
479 SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C20:0; MUFA: sum of C16:1,
480 C18:1 and C20:1; PUFA: sum of all C18:2 and C18:3.

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Table 4b. Homoegenous groups as determined by Tukey's HSD Test (*p-value* < 0.05) for C18's and major groups of fatty acids (% of total fatty acids) in *L. casei* fermented goat's milk as a function of lipid source and cold storage time

Factors	Lipid source			Time (days)				
	Control	Hydrolyzed	Tonalin®	0	1	14	28	35
C18:2	a	b	a	ab	b	a	ab	ab
C18:2 *c*9,*t*11-CLA	a	a	b	a	a	a	a	a
C18:2 *t*10,*c*12-CLA	a	b	c	a	a	a	a	a
ΣC18	a	c	b	a	ab	b	ab	a
SFA	c	a	b	ab	a	ab	ab	b
MUFA	a	b	b	a	b	b	ab	a
PUFA	a	c	b	ab	b	a	ab	ab

Abbreviation: *trans* (*t*), *cis* (*c*), conjugated linoleic acid (CLA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).
SFA: sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C20:0; MUFA: sum of C16:1, C18:1 and C20:1; PUFA: sum of all C18:2 and C18:3.

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Figure 1. The effect of storage time on *S. thermophilus* (St) counts estimated in M17 agar and *Lactobacillus* spp. (Lb) counts estimated in MRS agar of goat's yogurts with different lipid sources [control (C), yogurt with hydrolyzed sunflower oil (H) and yogurt with Tonalin® (T)].

Figure 2. The effect of storage time on *L. casei* counts estimated in MRS agar of goat's *L. casei* fermented milk with different lipid sources [control (C), yogurt with hydrolyzed sunflower oil (H) and yogurt with Tonalin® (T)].

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