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

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ABSTRACT

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

1. Introduction

Currently, one of the primary challenges in the food industry is to promote the development of healthy and sustainable foods. The food industry aims to generate fewer waste products and to valorise the co-products generated during food production and processing. Furthermore, these new foods should help reduce the risk of several illnesses, particularly non-communicable chronic diseases associated with modern lifestyles, such as diabetes and hypertension, important public health problems worldwide responsible for widespread morbidity and mortality. For example, 1.5 million deaths are directly attributed to diabetes each year (World Health Organization (WHO), 2024)). Over the past few decades, both the incidence and prevalence of these diseases have consistently risen.

In this context, being able to identify foods or ingredients with specific biological value (namely rich in antioxidants, antidiabetic compounds, antihypertensive agents or prebiotics, among others) to combat such prevalent health conditions, such as diabetes and hypertension, is crucial. Oxidative stress plays a crucial role in the development and progression of both these illnesses, and natural antioxidants present in certain foods may help neutralize harmful free radicals, reducing oxidative damage to cells and tissues (Chaudhary et al., 2023). In addition, certain bioactive compounds found in foods have been found to exhibit antidiabetic effects contributing to better glycemic control and reduced diabetes risk or antihypertensive properties which may help manage blood pressure and overall cardiovascular health. Not less important, is gut health, increasingly recognized as a key factor in preventing and managing chronic diseases. Prebiotics and non-digestible food compounds contribute to this context by selectively enhancing the growth of probiotics and supporting human health

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through nutrient enrichment, as well as by modulating beneficial gut microbiota and the immune system. Consuming foods rich in prebiotic compounds supports gut health and may indirectly impact diabetes and hypertension management (Megur et al., 2022). Taking into account the complexity and variety of the chemical components present in different food types, and the diversity of interactions in the biochemical networks and biological systems, metabolomic approaches will be essential in advancing nutritional food research (Emwas et al., 2021).

For millennia, dates (*Phoenix dactylifera*) have been a staple in the diets of inhabitants of different regions (mainly Arabian Peninsula and the Sahara Desert in North Africa) where local cultures have developed extensive knowledge of the benefits of dates, recognizing them as a rich source of essential nutrients which is highly influenced by variety or cultivar and growth conditions and areas (Alsuhaymi et al., 2023). The production and marketing of fresh dates in Spain has significantly increased due to promotional campaigns highlighting their growth characteristics. The Confitera cultivar, autochthonous and well-suited to the unique soil and climate conditions of the European oasis, demonstrates eco-efficient growth and supports sustainable production. Additionally, promotional campaigns have highlighted the nutritional and health benefits of dates (Fernández-López et al., 2022). Fresh dates contain valuable nutritional components such as sugars, dietary fibre, essential vitamins, and minerals, as well as bioactive compounds including polyphenolic compounds, anthocyanins, sterols, and carotenoids (Hussain et al., 2020; Muñoz-Bas et al., 2023). Furthermore, dates are a product deeply connected to the region, playing a vital role in the development of the local economy and serving as a prime example of a circular economy.

However, their commercialization leads to a substantial amount of coproducts, with around 30 % of dates being discarded due to low-quality classification based on factors such as size, colour, insects, or natural damages among others (Fernández-López et al., 2022) which usually end up as waste to be disposed of, with the consequent environmental risk. Various value-added products, such as rich fibre concentrates, date powder, date paste, date extracts and date juice, have been obtained from date co-products using environmentally friendly processes. These products have specific characteristics that make them suitable as functional ingredients in the food industry (Muñoz-Tebar et al., 2023). This approach has the potential to greatly minimize food waste in alignment with the UN's sustainable development objectives and support the circular economy.

Date juice and date powder are two of these value-added ingredients obtained from date co-products applying simple and environmentally friendly technologies (Muñoz-Bas et al., 2024). Both have different appearances, physicochemical properties, and compositions, including moisture, sugars, dietary fiber, organic acids, minerals and bioactive compounds. This provides them with significant flexibility for integration into various food matrices, based on the technological goals to be met and the specific attributes of the new product being created. For instance, date juices could be utilized in crafting fresh fruit beverages, syrups and liquid caramel. It could also be used as an ingredient in the development of fermented meat products, as well as in bakery, confectionery and reconstitution medium for the dairy industry. Similarly, date powder could be used as an ingredient for the development of bakery, pastry, and meat products (Muñoz-Bas et al., 2024). Considering the multitude of food applications of date juice and powder, the evaluation of their biological activities, such as antioxidant antidiabetic, antihypertensive and prebiotic activities, will be of great importance for demonstrating their potential beneficial effects on human health.

Based on the above rationale, the aim of this work was to provide, for the first time, an in-depth characterization of the chemical composition (total soluble solids (TSS), total dietary fibre (TDF), total sugars, proteins and fat, moisture, ash and total phenolic content) and of associated biological activities (antioxidant, antidiabetic, antihypertensive and prebiotic properties) of date juice and date powder, which are added-value ingredients derived from date coproducts.

2. Materials and methods

2.1. Preparation of high added-value ingredients from date co-products

Date co-products (non-commercial or discard dates due to externally damaged, undersized, etc.) from the industrialization of fresh dates (Confitera cv.) harvested at the tamar stage from the Elche Palm Grove (Elche, Alicante, Spain) were provided by the Catedra Palmeral d'Elx (UMH, Alicante, Spain). These co-products were transported under refrigerated conditions (6 ± 2 °C) to the Food Pilot Plant of the Orihuela Polytechnic School (EPSO) of the Miguel Hernández University and immediately processed following the procedure described by Muñoz-Bas et al. (2024) to obtain date juice and date powder, applying only physical treatments. Briefly, the dates were pitted and grounded and the resulted paste was soaked several times with distilled water- Then the juice date was obtained by pressing through cotton filter clothes and the powder date by drying the remaining solid part in an oven (60 ºC, 24 h). Date juice was packaged in opaque glass containers and frozen at -18 °C, while the date powder was vacuum-packed in plastic bags protected from light. Part of the conditioned products (date powder and date juice) were analysed for chemical composition whereas a second part was transported to the laboratories of the Catholic University of Porto (Porto, Portugal) to carry out the remaining chemical analyses (total phenolic content) and the assessment of the antioxidant, antidiabetic, antihypertensive and prebiotic biological activities.

2.2. Chemical analysis of high added-value ingredients

Date powder was analyzed in triplicate for moisture (AOAC 925.45), total protein (AOAC 981.10), total fat (AOAC 991.36), ash (AOAC 923.03), and total dietary fiber (AOAC 985.29) contents following AOAC methods (AOAC 2006). Moisture content was measured by drying 2 g of sample in a vacuum oven. Protein content was estimated from the analysis of the nitrogen content through the Kjeldahl method (Bloc digest 12 (Selecta, Barcelona, Spain), and Kjeltec 8400 analyzer unit (Foss Hillerod, Denmark), using a conversion factor of 6.25. The ash content was assessed by the incineration of 2 g of sample at 550 ºC until the total elimination of organic matter. The fat content was determined according to the Soxhlet extraction principle using a semiautomatic extractor (SOXTHERM SOX, Gerhardt GmbH & Co., Königswinter, Denmark). Total dietary fiber was assessed by the enzymatic-gravimetric method using the GDE enzymatic digester and CSF filtration system (Velp Scientifica, Usmate, Italy). Total sugar content was calculated by difference, subtracting the sum of the other components (moisture, protein, fat, ash, and dietary fiber) from the total (100 %).

Total soluble solids (TSS) content of date juice was determined using a digital refractometer Milwaukee MA 871 (Milwaukee electronics, Milwaukee, WI, USA) and expressed as ^{0}Brix.

2.3. Date powder extracts preparation

The date powder extracts were prepared according to the method described by Hung et al. (2011), with minor modifications. Initially, 20 mL of 80 % ethanol, previously prepared by diluting absolute ethanol (VWR, PA, USA), were added to 2 g of the date powder sample. The solution was homogenised by agitation in an orbital shaker (Orbital Shaker Wiggenhouse, Berlin, Germany) for 20 min at 200 rpm at a temperature of 30 °C, followed by sonication (Bath sonicator, Bandelin, Berlin, Germany) for 10 min. Subsequently, the sample was subjected to centrifugation at 3850×g for 5 min at 20 °C (Hettich Universal 320R Centrifuge, Andreas Hettich GmbH & Co.). The supernatant was collected in a new 50 mL tube and the procedure was repeated by adding 10 mL of 80 % ethanol. The samples were concentrated using a rotavapor (Buchi, Flawil, Switzerland). Each sample was subjected to a 30 min exposure to the rotavapor at a temperature of 45 °C and a pressure of 100 atm. A final volume of 5 mL of sample was obtained.

2.4. Total phenolic content determination

The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method, following the procedure described by Coscueta et al. (2018), with minor amendments. A standard curve of gallic acid (0.025–0.200 mg/mL) was created to present the findings in mg gallic acid equivalents per mL of sample (mg GAE/mL). Using a 96-well microplate, 30 μL of each sample (or suitable dilution), 100 μL of Folin-Ciocalteu reagent (20 % v/v) (Merck KGaA, Darmstadt, Germany), and 100 μL of anhydrous sodium carbonate solution (7.4 % w/v) were pipetted into each well. The microplate was then incubated, wrapped in aluminium foil, for 30 min at 25 °C in darkness. The resulting mixture was measured at 765 nm using a multi-detection plate reader (Synergy H1, VT, USA) with Gen5 software. All measurements were conducted in triplicate.

2.5. The 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging assay

The antioxidant activity was evaluated using the 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay (ABTS) as outlined by Gonçalves et al. (2009), with minor adjustments. Initially, the ABTS working solution's concentration was modified to achieve an absorbance of 0.70 (± 0.02) at 734 nm. To create the Trolox solution, 0.0125 g of Trolox (Sigma-Aldrich, MO, USA) was measured and dissolved in 1 mL of methanol (Fischer Chemical, MA, USA), then diluted to 50 mL final volume with deionised water. The results were expressed in Trolox equivalents, utilising a standard curve (25 μM-175 μM). For the assay, 20 μL of Trolox or sample and 180 μL of ABTS solution were added to each well of a 96-well microplate. The microplate was subsequently incubated for 5 min at 30 °C and the absorbance was measured at 734 nm using a Multi-detection plate reader (Synergy H1, VT, USA). All analyses were conducted in triplicate.

2.6. Prebiotic activity

Date juice and date powder were assessed for their potential to promote growth and metabolic activity of different probiotic strains. For date juice, being a liquid matrix, the prebiotic potential was assessed using the 96-well microplate method (microscale) determining cell growth via absorbance, while for date powder, being a solid matrix, the prebiotic potential was assessed at macroscale determining cell growth by viable cell numbers enumeration using colony-forming unit (CFU) counts.

2.6.1. Growth media preparation

The basal medium used for the evaluation of the bifidogenic potential of date juice and date powder was Man-Rogosa-Sharpe broth (MRS) prepared by mixture of the different components to allow carbon source substitution. The detailed composition of MRS broth is described in Table 1.

From this basal medium the following media were prepared:

1. MRS basal broth without glucose, as negative control
2. MRS with 2 % (w/v) glucose (Fluka, Charlotte, NC, USA), as positive control
3. MRS with 2 % (w/v) fructooligosaccarides (FOS) (Orafti, Oreye, Belgium), as positive prebiotic control.
4. MRS with 2 % and 10 % (v/v) of date juice
5. MRS with 2 % and 6 % (v/v) of date powder

The two concentrations of date juice and date powder tested were established based on the respective total sugars content, to approach that found in MRS medium. In the case of *Bifidobacterium* stains all media were subsequently supplemented with filter-sterilized 0.5 g/L of l-cysteine·HCl (Alfa Aesar, Kandel, Germany).

Table 1
Detailed composition of Man-Rogosa-Sharpe basal medium.

Compound	Source	Concentration (g/L)
Tryptone	Sigma-Aldrich (St. Louis, MI, USA)	10
Meat extract	Merck (Darmstadt, Germany)	8
Yeast extract	Biokar Diagnostics (Allone, France)	4
Di-potassium hydrogen phosphate	Merck (Darmstadt, Germany)	2
Tween 80	Merck (Darmstadt, Germany)	1
Sodium acetate	Merck (Darmstadt, Germany)	5
Ammonium citrate tribasic	Sigma-Aldrich (St. Louis, MI, USA)	2
Magnesium sulfate	Merck (Darmstadt, Germany)	0.2
Manganese sulfate	Sigma-Aldrich (St. Louis, MI, USA)	0.04
Carbon source	-	20

2.6.2. Bacterial growth

For the microplate assay, 11 probiotic strains were selected for screening including, *Lactobacillus acidophilus* KI (CSK, Ede, Netherlands), *Lacticaseibacillus paracasei* L26 (Christian (Chr) Hansen, Hørsholm, Denmark), *Lacticaseibacillus rhamnosus* R11 (Lallemand, Montréal, QC, Canada), *Lacticaseibacillus casei* 01 (Chr. Hansen, Hørsholm, Denmark), *Lactobacillus acidophilus* La-5 (Chr. Hansen, Hørsholm, Denmark), *Lactiplantibacillus plantarum* 299v (Probi AB, Lund, Sweden), *Bifidobacterium animals* subspecies *lactis* BB-12® (Chr. Hansen, Hørsholm, Denmark), *Bifidobacterium breve* NCIMB, *Bifidobacterium animalis* Bo (CSK, Ede, Netherlands), *Bifidobacterium longum* BG3 (Cell Biotech, Hellerup, Denmark) and *Bifidobacterium animalis* BLC (DSM Food Specialties, Moorebank, Australia).

The probiotic strains with the best performance in the microplate assay were selected for the sequential macroplate assay, namely: L. *casei* 01, L. *rhamnosus* R11, *B. breve* NCIMB, *and B. animalis* BLC.

For each experiment, the bacterial strains were reactivated in the appropriate broth for 24 h. The lactobacilli strains were grown under aerobic conditions, while the bifidobacteria strains were grown under anaerobic conditions (85 % N_2, 5 % H_2, and 10 % CO_2), achieved using a Whitley A35 HEPA anaerobic workstation incubator (Bingley, United Kingdom), after thawing a glycerol stock. For the growth of *Lactobacillus* strains MRS medium was used, while for the growth of *Bifidobacterium* stains MRS medium was subsequently supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl (Alfa Aesar, Kandel, Germany). For all strains, a single sub-culturing step was carried out under identical growth conditions, with a final incubation volume of 10 mL of MRS (supplemented with 0.05% cysteine for Bifidobacterium) and 1 % (v/v) cell inoculation. The initial colony-forming unit (CFU) count in each inoculum suspension was determined by preparing serial dilutions in PBS (Sigma-Aldrich, St. Louis, MI, USA). Subsequently, 10 μL of each dilution were plated in triplicate on suitable media. Agar plates were incubated at 37 °C for 48 h under anaerobic or aerobic conditions for bifidobacteria and lactobacilli strains, respectively. Following incubation, CFU enumeration was conducted, and the results were expressed as mean ± standard deviation CFU/mL for bacterial suspensions.

2.6.3. Screening of bacteria growth via microplate assay

The screening of the bacterial growth for the 11 probiotic strains via the microplate assay was performed according to Sousa et al. (2015), with slight modifications. Each previously prepared medium (refer to Section 2.6.1) was inoculated with the corresponding probiotic strain at a concentration of 2 % (v/v), in triplicate. Subsequently, 250 μL of the probiotic incorporated-growth medium was transferred to each corresponding well of a 96-well microplate. To prevent the presence of oxygen, 50 μL of autoclave-sterilized liquid paraffin (Merck, Germany) was added to the wells containing bifidobacteria strains. Cellular growth was

monitored throughout 24 h by measuring the optical density (OD) of the cultures at 655 nm using a 680 Microplate Reader from Bio-Rad (Hercules, CA, USA) in conjunction with Microplate Manager 5.2.1 Software. A negative control was established using MRS without glucose, while a growth control and a positive control were established by supplementing the MRS broth with glucose and FOS, respectively. The specific growth rates were determined by calculating the slope of the trend line and the absorbance values in the log phase of the growth curves. The maximum growth (maximum absorbance) was assessed to compare the results of the different growth conditions.

2.6.4. Evaluation of bifidogenic potential via determination of viable cell numbers

In the case of the date powder, its bifidogenic potential was assessed by evaluating the growth behavior of the four previously selected commercial probiotic strains (refer to Section 2.6.3). Their growth and acidification capacities in each of the five previously described growth media was measured via enumeration of viable cell numbers and measurement of pH evolution, following the procedure outlined by Sousa et al. (2015), with minor modifications. Bacterial metabolism was evaluated by measuring pH using a pH-meter (Crison Instruments, Barcelona, Spain). Each medium was inoculated with the corresponding probiotic strain at a concentration of 2 % (v/v). The inoculated media were then transferred to sterile 2mL Eppendorf tubes (in triplicate), and incubated at 37 ºC for 24 h under aerobic and anaerobic conditions for lactobacilli and bifidobacteria strains respectively (according to conditions mentioned in 2.6.2). To ensure anaerobic conditions for the *Bifidobacterium* strains, the media were supplemented with filter-sterilized 0.5 g/L of l-cysteine·HCl. Sampling was performed at 0, 3, 6, 10 and 24 h. Decimal dilutions were prepared in PBS at each sampling interval, and 10 µL of each dilution were plated thrice on suitable media. Following incubation, CFU counting was conducted, and results were reported in CFU/mL.

2.7. Antidiabetic activity (α-glucosidase inhibition assay)

The antidiabetic activity was assessed by measuring the α-glucosidase inhibitory activity, following a modified version of the method described by Kwon et al. (2008). Initially, 50 µL of sample was combined with 100 µL of α-glucosidase solution (1.0 U/mL) diluted in 0.1 M phosphate buffer (pH 6.9) per well. The microplate was then incubated at 25 °C for 10 min. Following incubation, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer was added to each well. The mixtures were subsequently incubated at 25 °C for 5 min, and absorbance was measured at 405 nm using a Multi-detection plate reader (Synergy H1, VT, USA). For assay efficacy control, 50 µL of buffer solution served as a negative control, whilst 50 µL of acarbose at 10 mg/mL concentration was used as a positive control. All measurements were conducted in triplicate. The α-Glucosidase inhibition percentage was calculated using the following formula:

$$\alpha - Glucosidase\ inhibition\ (\%) = \left(\frac{\Delta Abs_{control} - \Delta Abs_{sample}}{\Delta Abs_{control}}\right) x100$$

2.8. Antihypertensive activity (Angiotensin-I converting enzyme (ACE)-inhibitory activity assay)

The antihypertensive effect was evaluated via an ACE-inhibitory activity test as outlined by Sentandreu and Toldra (2006), with slight changes. Each well received 40 µL of ultrapure water or ACE working solution (42 mU/mL), and the final well volume was brought to 80 µL using ultrapure water. Then, 160 μL of substrate solution (0.45 mM) was added and the resulting mixture incubated at 37 °C for 30 min to allow the enzyme reaction to occur. Throughout the 30 min, the resulting fluorescence was measured with excitation and emission wavelength at 350 and 420 nm, respectively, using a Multi-detection plate reader (Synergy H1, VT, USA). All measurements were performed in triplicate. The ACE inhibitory activity was calculated using the following formula:

$$iACE(\%) = ((F_{CTL} - F_{BLK}) - (F_{SPL} - F_{SPLB})) * \frac{100}{F_{CTL} - F_{BLK}}$$

2.9. Statistical analysis

Data from this investigation were analyzed using SPSS software version 17.0 (SPSS; Chicago, IL, USA). These data were expressed as the mean ± standard derivation (SD) of replicates. All experiments were made in triplicate. Parametric tests were conducted on the data, which were found to follow a normal distribution according to the Shapiro-Wilk test (normality test). A t-Student for independent samples test was performed in order to conduct a statistical comparison between date juice and date powder. Statistical differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition

The nutritional value of fruit juices is due to their content in carbohydrates (mainly sugars), minerals, vitamins, organic acids, and bioactive compounds, which amounts depend on the original fruit, extraction process and preservation method applied because some of these compounds are highly susceptible to degradation. Dates are no exception, and the co-products date juice and powder, obtained during the processing of fresh dates will vary in composition depending on several factors including the separation process, the maturity of the dates, the presence of enzymes and their activity levels, extraction temperature and the drying process. For example, the drying process will influence the date powder composition, including sugar concentration, antioxidants, and dietary fiber content.

Indeed, and according to the reported results the high content of soluble sugars in date fruits (Muñoz-Bas et al., 2023) was easily extracted and passed into the water during the elaboration process of date juice. The date juice showed a high Total Soluble Solid (TSS) content (18.3–19.5 ^{o}Brix) which is in agreement with TSS in freshly prepared date juice (Kulkarni et al., 2010). The main component found in date powder was Total Dietary Fibre (TDF) (66.0 ± 1.4 g/100 g), followed by total sugars (18.7 ± 0.8 g/100 g), total protein (6.8 ± 0.7 g/100 g), moisture (5.7 ± 1.0 g/100 g), ash (1.7 ± 0.3 g/100 g) and total fat (1.2 ± 0.7 g/100 g). Date fruits are considered excellent sources of carbohydrates, comprising 60 % to 80 % of their composition, which includes both soluble sugars and dietary fiber (Muñoz-Bas et al., 2023; Stojanovska et al., 2023). The TDF content in date fruits ranges between 5.3 and 13.4 % depending on cultivar, growth conditions and ripening stage, being insoluble fiber the main fraction (77–90 %, mainly lignin) (Stojanovska et al., 2023). In-depth studies of date fruit fibers have indicated that dates are a source of soluble dietary fibers, including fructan, pectin, galactomannan, arabinoxylan, and β-glucan, with varying levels of these components found among different cultivars (George et al., 2020; Dhahri et al., 2023).

3.2. Prebiotic activity

The impact of prebiotics and probiotics on human nutrition and health is well-established (Yadav et al., 2022). Both have been commonly used as functional ingredients in the development of functional foods. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), prebiotics are “substrates that are selectively utilized by host microorganisms conferring a health benefit”. This group includes compounds of very different chemical structures, such as oligosaccharides, polyphenols, and even polyunsaturated fatty

acids that have been converted to their respective conjugated forms. Prebiotics have protective effects on the gastrointestinal system by positively modulating gut bacteria. Additionally, they can reduce blood lipid levels, improve insulin resistance, and enhance mineral bioavailability (Davani-Davari et al., 2019; Faustino et al., 2023; Gibson et al., 2017).

3.2.1. *Date juice prebiotic activity*

Figs. 1 and 2 illustrate the growth curves of the six lactobacilli strains and the five *Bifidobacterium* strains in the different MRS-supplemented media, respectively, while Table 2 lists the specific growth rates for each strain in each growth medium containing different carbon sources. Positive and negative control experiments were conducted to validate the experimental setup. Positive control assays utilized modified MRS broth supplemented with glucose and fructooligosaccharides (FOS) as carbon sources. Conversely, negative control experiments employed MRS broth devoid of any carbon source. As anticipated, the negative control medium (MRS without glucose) exhibited significantly restricted microbial growth for all the strains assayed (low specific growth rates, Table 2), since there is no sufficient carbon source available to allow normal growth of lactobacilli and *Bifidobacterium* strains.

The examination of Fig. 1 and Table 2 showed variations in the growth patterns of the six lactobacilli strains based on the carbon source under study. All strains exhibited good growth in glucose, although at different rates, with L. *plantarum* 299v (0.833 h^{-1}) showing the best growth performance, followed by L. *rhamnosus* R11 (0.752 h^{-1}), L. *paracasei* L26 (0.611 h^{-1}) and L. *casei* 01 (0.601 h^{-1}) (Table 2). Conversely, L. *acidophilus* La5 and L. *acidophilus* Ki reported lower specific growth rates (0.423 h^{-1} and 0.349 h^{-1}, respectively). In general, the maximum absorbance values followed this trend, the L. *acidophilus* strains reached OD_{600nm} levels of 0.9 and 1.4 for L. *acidophilus* Ki and L. *acidophilus* La5, respectively, compared to OD_{600nm} levels of 2.0–2.1 for the other lactobacilli strains studied (Fig. 1). The addition of FOS as the sole carbon source did not enhance the growth of the six lactobacilli strains as much as glucose; specific growth rates were either similar (as reported for the two L. *acidophilus* strains; $p > 0.05$) or 0.10–0.25 units lower ($p < 0.05$; Table 2). The maximum OD_{600nm} levels followed the same trend. Date juice, especially at a concentration of 10 % (w/v), demonstrated a more potent growth-promoting effect compared to FOS and was comparable to the effect of glucose on the growth of all lactobacilli strains examined except for the two L. *acidophilus* Ki and La5 strains. In this latter case growth parameters were improved both in terms of specific growth rates and OD_{600nm} levels. Significant variances between the two concentrations of date juice were noted ($p < 0.05$), particularly in terms of maximum OD_{600nm} levels, with the most notable differences observed in the two L. *acidophilus* Ki and La5 strains. Nonetheless, it is important to emphasize that incorporating media with 2 % date juice led to increased growth rates compared to using FOS for all tested strains.

Based on the above, it can be inferred that in order to achieve optimal growth of lactobacilli species, 10% date juice supplementation is recommended. The higher the percentage of date juice, the higher the concentration of available carbohydrate sources, namely sugars. A higher concentration of available sugars leads to improved growth rates

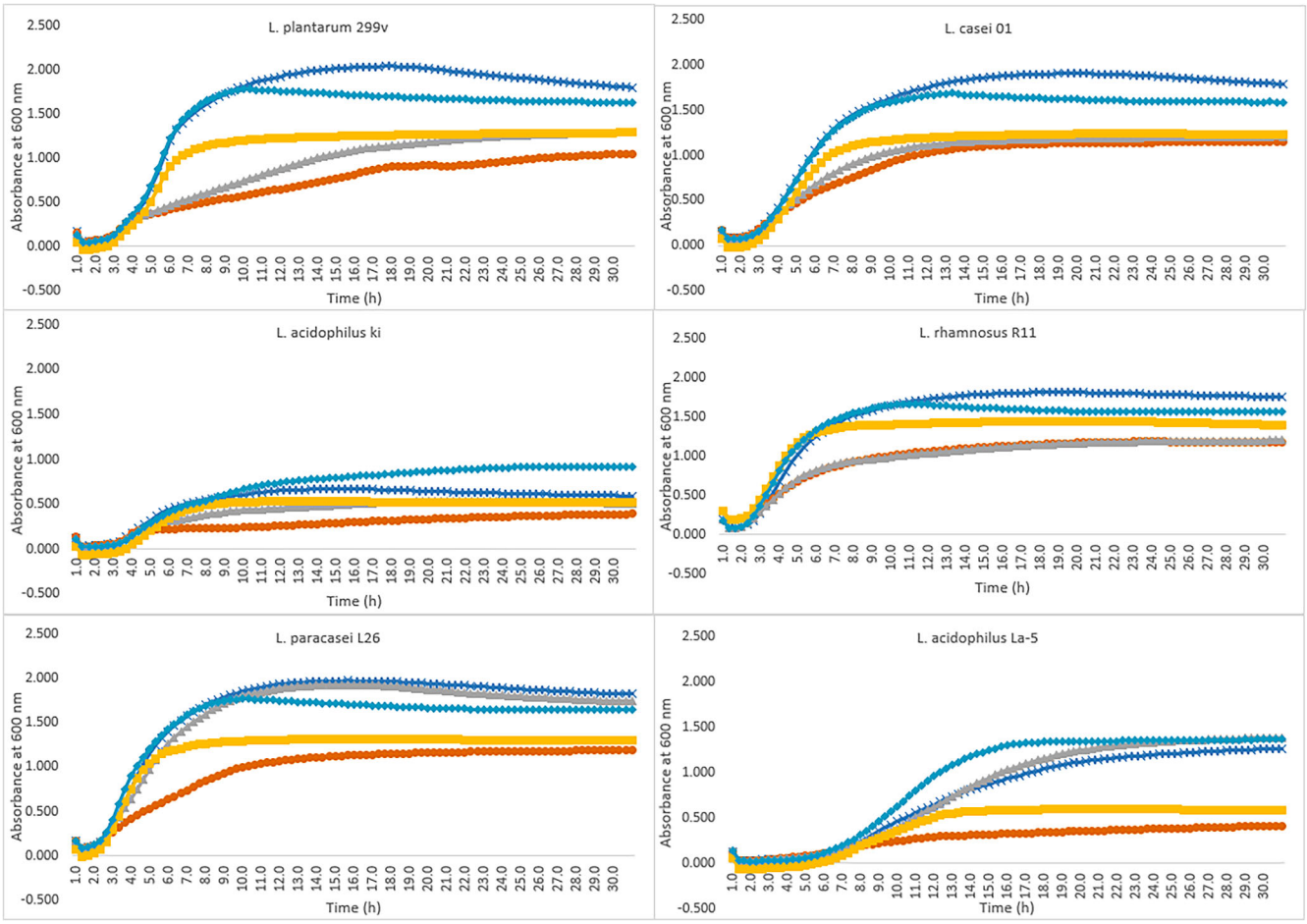

Fig. 1. Growth curves for Lactobacillus strains (L. *plantarun 299v,* L. *acidophilus ki,* L. *acidophilus La-5,* L. *paracasei L26,* L. *casei 01, and* L. *rhamnosus R11)* grown in different culture media [basal MRS medium without glucose (orange), MRS with glucose (dark blue), MRS with FOS (gray) MRS with 2% date juice (yellow) and MRS with 10% date juice (light blue) determined by measuring OD of the cultures at 600 nm over a 30 h-period.

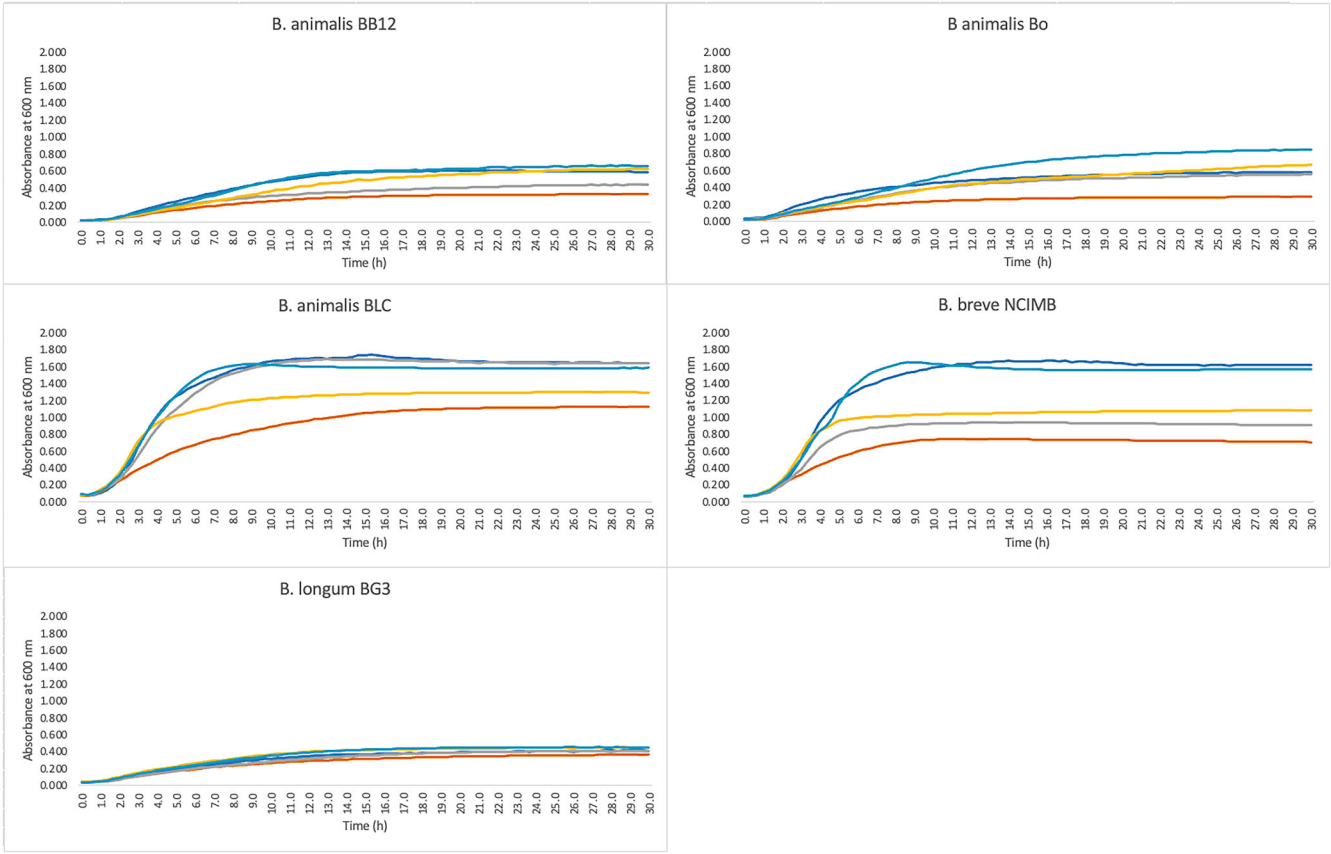

Fig. 2. Growth curves for Bacillus strains (*B. animalis BB12, B. animalis Bo, B. animalis BLC, B. breve NCIMB and B. longum BG3)* grown in different culture media [basal MRS medium without glucose (orange), MRS with glucose (dark blue), MRS with FOS (gray) MRS with 2% date juice (yellow) and MRS with 10% date juice (light blue) determined by measuring OD of the cultures at 600 nm over a 30 h-period.

Table 2
Specific growth rates for the lactobacilli and *Bifidobacterium* strains tested in the different MRS media, with different carbon sources including different date juice (DJ) concentrations (2 and 10 %).

Strain	Specific growth rate (h^{-1})				
	Carbon Source %(w/v)				
	Glucose	None	FOS	DJ 2%	DJ 10%
Lactobacilli					
L. *acidophilus* Ki	0.423	0.265	0.400	0.558	0.480
L. *acidophilus* La5	0.349	0.203	0.307	0.404	0.437
L. *rhamnosus* R11	0.752	0.420	0.568	0.607	0.752
L. *paracasei* L26	0.611	0.339	0.543	0.664	0.650
L. *casei* 01	0.601	0.391	0.515	0.621	0.627
L. *plantarum* 299v	0.833	0.447	0.556	0.754	0.838
Bifidobacterium					
B. animalis BB12	0.596	0.483	0.572	0.523	0.568
B. animalis Bo	0.421	0.403	0.441	0.324	0.394
B. animalis BLC	0.715	0.578	0.604	0.647	0.690
B. breve NCIMB	0.679	0.419	0.616	0.631	0.655
B. longum BG3	0.446	0.429	0.456	0.466	0.472

for bacterial species. Nevertheless, it is worth noting that exceeding the 10 % threshold of date juice may not necessarily result in higher growth rates, as excessively high sugar concentrations could inhibit bacterial growth (Cai et al., 2021; Mizzi et al., 2020).

The growth patterns of *Bifidobacterium* strains were found to be different depending on the carbon source being studied (Table 2; Fig. 2). *Bifidobacterium animalis* BLC exhibited the highest specific growth rates, regardless of the carbon source, while *B. animalis* Bo showed the lowest values (Table 2). This observation was supported by the maximum OD_{600nm} levels reached – 1.8 for *B. animalis* BLC and 0.6 for *B. animalis* Bo (Fig. 2). *Bifidobacterium longum* BG3 also displayed low specific growth rates, which were consistent across different carbon sources; its growth was equally influenced by date juice, glucose, and FOS (the latter being positive controls). With the exception of *B. animalis* Bo, all *Bifidobacterium* strains exhibited similar maximum OD_{600nm} levels when grown in MRS supplemented with 10 % (w/v) date juice compared to glucose. Additionally, growth in MRS supplemented with 2 % (w/v) date juice closely resembled that achieved with FOS for all strains except *B. animalis* Bo.

As seen in Section 3.1, date juice consists mainly of water-soluble solids that are transferred during the production of the co-product. These solids are mainly soluble sugars such as glucose and fructose, which represent 38.2–44.7 % and 36.8–40.1 % of the total dry mass, respectively (Muñoz-Bas et al., 2024; Kamal-Eldin et al., 2020). Supplementing the basal medium with 10% date juice may result in a higher concentration of sugars in the medium compared to the control basal medium containing 20 g/L. The higher sugar concentration will consequently result in higher bacterial growth rates.

3.2.2. *Date powder prebiotic activity*

Fig. 3 displays the growth curves of the four selected probiotic bacteria (*Lacticaseibacillus rhamnosus* 11, *Lactobacillus casei* 01, *Bifidobacterium breve* NCIMB, and *Bifidobacterium animalis* BLC) to assess the growth-factor potential of date powder at two different concentrations (2 and 6 % (w/v)) over a 24 h period as determined by viable cell enumeration.

By analyzing Fig. 3, it can be inferred that the use of 2 % (w/v) date powder exhibited a prebiotic potential. The media supplemented with date powder (2 % (w/v)) had higher viable cell numbers (CFU/mL)

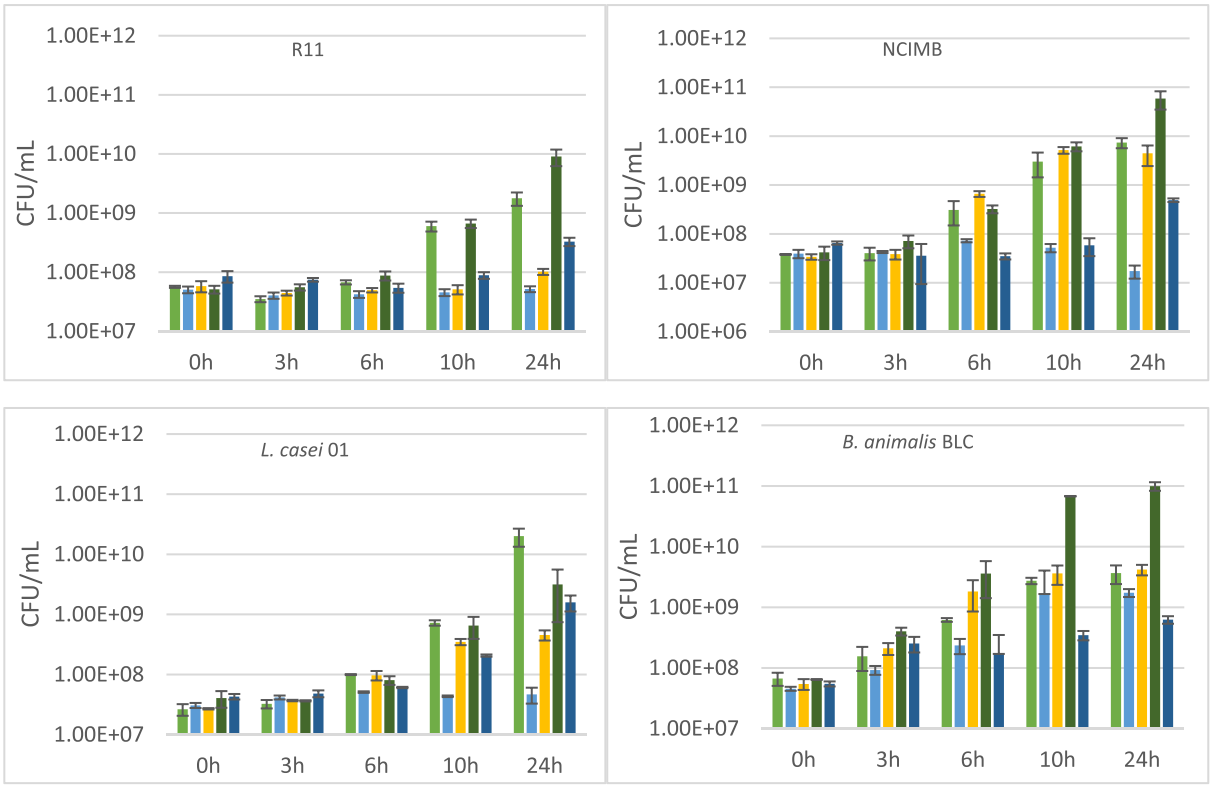

Fig. 3. Evaluation of the growth of selected probiotic bacteria *(Lacticaseibacillus rhamnosus 11, Bifidobacterium breve NCIMB, Lactobacillus casei 01, Bifidobacterium animalis BLC)* in different culture media (light green: MRS with glucose; light blue: MRS without glucose; yellow: MRS with FOS; dark green: MRS with 2% date powder; dark blue: MRS with 6% date powder).

compared to MRS-FOS (prebiotic positive control) and MRS with glucose (positive control) after 24 h of incubation for all the probiotic bacteria tested except for L. *casei* 01. It is noteworthy that the growth promotion of L. *casei* 01 was comparable between the two media supplemented with date powder (2 % and 6 % (w/v)) and even higher than that registered for MRS-FOS. However, when this strain was incubated in MRS with glucose, the highest viable cell numbers were achieved at 24 h. The *Bifidobacterium* strains (*B. breve* NCIMB and *B. animalis* BLC) showed higher viable cell numbers (5.50×10^{10} CFU/mL and 9.77×10^{10} CFU/mL, respectively) than L. *casei* 01 or L. *rhamnosus* 11 after a 24 h incubation period with 2% date powder. When compared to the positive control FOS, the same strains did not reach such high numbers, with viable cell counts of 1.10 and 1.37 log cycles lower at the same 24 h time point, respectively. Notably, the L. *rhamnosus* R11 strain exhibited a particularly pronounced difference, reaching 1.95 log cycles higher when date powder was used.

Typically, food sources of prebiotic compounds contain high levels of carbohydrates, specifically non-digestible carbohydrates such as dietary fibers. As observed in Section 3.1., date powder is a valuable source of dietary fibers, with a TDF threshold of 66.0 ± 1.4 g/100 g. By having high amounts of TDF, date powder is a potential natural source of prebiotic compounds. In light of the aforementioned considerations, it was anticipated that the experimental conditions containing 2 % and 6 % (w/v) date powder would facilitate the growth of the selected probiotic strains. A 2017 study, which explored the prebiotic potential of date seeds on stimulating the growth of lactobacilli strains, yielded comparable results. The presence of date seeds stimulated the growth of lactobacilli strains with values comparable to those observed in the positive controls (Al-Thubiani and Khan, 2017). It is important to note that the prebiotic effect of the developed date powder may result from the combination of fermentable water-soluble simple carbohydrates and dietary fibers.

It should be emphasized that the condition containing 6 % (w/v) date powder yielded lower CFU/mL values compared to 2 % (w/v) date powder, suggesting that increasing the concentration of date powder may not necessarily improve the prebiotic potential. A similar trend was observed in the evaluation of yacon tuber flour's prebiotic potential; the addition of 1 % (w/v) yacon flour revealed better growth outcomes compared to the addition of 2 % (w/v) (Sousa et al., 2015). Therefore, based on the abovementioned findings, 2 % (w/v) date powder appears to be an optimal concentration for promoting the growth of the selected probiotic strains. Furthermore, Fig. 4 corroborates these results by illustrating the temporal evolution of pH during the growth of the four selected probiotic bacteria over a 24 h incubation period. As anticipated, pH levels exhibited a decline over the assessment period, indicating fermentative activity by the probiotic bacteria. This decline can be attributed to the conversion of available sugars into corresponding acids through fermentative activity; growth and acidification activity were well correlated.

In light of the aforementioned considerations, it can be reasonably inferred that 2 % (w/v) date powder exerts a prebiotic effect on the selected probiotic strains.

3.3. Total phenol content and antioxidant activity

Phenolic compounds, the most abundant secondary metabolites in plants, are characterized by a common chemical structure consisting of an aromatic ring with one or more hydroxyl substituents. These compounds are highly effective in neutralising free radicals and exhibit antioxidant properties. Furthermore, phenolic compounds offer a range of health benefits including antibacterial, antihyperlipidemic, anticancer, antioxidant, cardioprotective, neuroprotective, and antidiabetic properties (Al Manary, 2022; Khoddami et al., 2013; Lin et al., 2016). There is a strong correlation between the phenolic compound content and antioxidant activity, meaning that higher levels of phenolic compounds are linked to greater antioxidant activity (Muflihah et al., 2021).

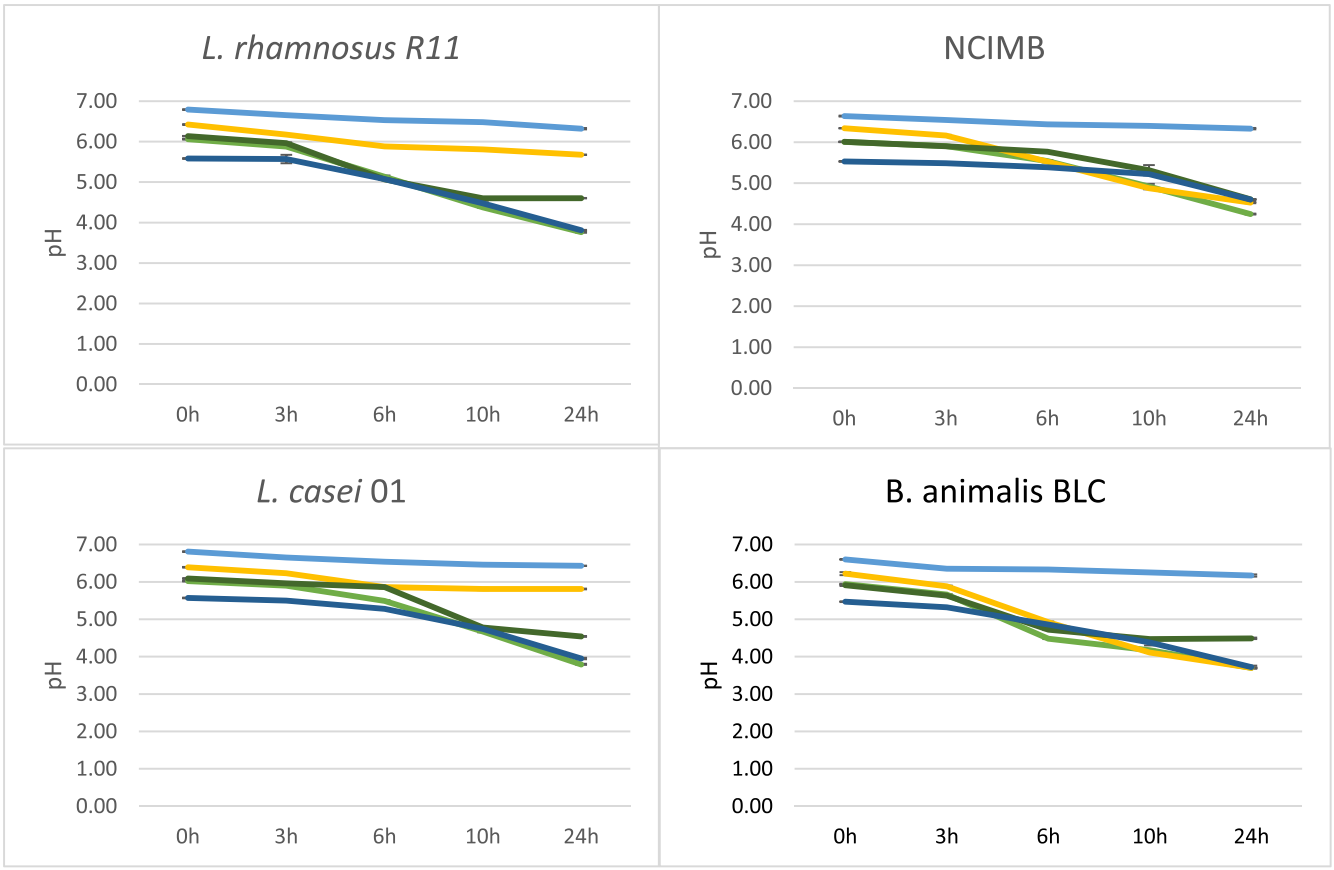

Fig. 4. pH evolution during the growth of selected probiotic bacteria *(Lacticaseibacillus rhamnosus 11, Bifidobacterium breve NCIMB, Lactobacillus casei 01, Bifidobacterium animalis BLC)* in different culture media (light green: MRS with glucose; light blue: MRS without glucose; yellow: MRS with FOS; dark green: MRS with 2% date powder; dark blue: MRS with 6% date powder).

Table 3 shows the total polyphenol content (TPC) and the antioxidant activity of date juice and date powder. As anticipated, date powder (1.575 ± 0.028 mg GAE/g) exhibited higher TPC than date juice (0.146 ± 0.004 mg GAE/mL), resulting consequently in higher antioxidant activity (Table 3). The water extraction process involves the filtration of solid substances from the source water, resulting in the production of the final product. In this context, it is possible that phenolic compounds may remain bound to dietary fibre, resulting in a lower concentration of these compounds in the juice justifying the lower TPC content (Zhang et al., 2017). As previously stated, a correlation has been identified between TPC and antioxidant activity; an increase in TPC is accompanied by an increase in antioxidant activity, and vice versa (Kumar et al., 2014; Muflihah et al., 2021). In this context, date juice exhibited a relatively low antioxidant activity (702.060 ± 23.147 μmol of TROLOX equivalent/mL), which correlated with the low phenolic compounds content present, whereas date powder reported a high antioxidant activity (5823.026 ± 27.247 μmol of TROLOX equivalent/g) aligned with the higher TPC content (Table 3). The high antioxidant activity observed in the present study is in line with the findings of previous research, which has reported the potential antioxidant activity of date fruits (Allaith 2019; Rahmani et al., 2014). Date fruits are considered to be good sources of phenolic compounds (Alsuhaymi et al., 2023). Higher concentrations of phenolic compounds are mainly found in the pulp and in the seed fraction of the date fruit (Khatib et al., 2022). Date powder studied herein is mainly composed of grinded and dried fresh date pulp, resulting therefore in the high concentration of TPC observed in Table 3, namely 1.575 ± 0.028 mg GAE/g TPC, in date fruits ranges from 2.06 ± 0.06 to 6.53 ± 0.18 mg GAE/100 g DW (Ali Haimoud et al., 2016) to 753.30 mg GAE/100 g DW (Shahdadi et al., 2015), depending on several factors such as cultivar, growth conditions, ripening stage and extraction procedure among others (AlFaris et al., 2021; Bouhlali et al., 2017). A comparison of the obtained results with those reported in the literature, considering the final ripening stage, indicates that the date powder contains approximately one-third of the TPC found in the whole date fruit. Consequently, it can be inferred that approximately one-third of the TPC found in the date fruit is associated with the dietary fiber present in the date pulp.

Furthermore, it is important to note that the same factors responsible for the amount of TPC found in the date fruit are also responsible for the relative content of the different polyphenols found in date fruits, such as cinnamic and coumaric acids and their derivatives, including ferulic, sinapic, syringic, vanillic, gallic, caffeic, protocatechuic and dactilyferic acids, as well as flavonoid glycosides (luteolin, methyl luteolin, quercetin, and methyl quercetin), flavones, flavanols (catechin, epicatechin), flavaxanthin and anthocyanins (Jdaini et al., 2023).

Table 3
Total Phenolic content (TPC), antioxidant activity (ABTS assay), antidiabetic activity (α-glucosidase inhibition) and antihypertensive activity (inhibition of angiotensin-I-converting enzyme (ACE)) of date juice and date powder.

	Date juice	Date powder
TPC (mg GAE/X)	0.146 ± 0.004b (mg GAE/mL)	1.575 ± 0.028a (mg GAE/g)
ABTS	702.060 ± 23.147b (μmol of TROLOX equivalent/mL)	5823.026 ± 27.247a (μmol of TROLOX equivalent/g)
α-glucosidase inhibition (%)	76.34 ± 0.78b	87.62 ± 2.04a
ACE inhibition (%)	55.73 ± 4.92b	92.24 ± 0.73a

$n = 3$. a-b: Different letters in the same row indicates significant differences ($p < 0.05$).

3.4. Antidiabetic activity

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, which can lead to long-term damage to various organs in the body. According to recent data from the World Health Organization (WHO) (2024), approximately 422 million individuals worldwide are living with diabetes, with a significant proportion residing in low- and middle-income countries. Furthermore, this condition is directly responsible for an estimated 1.5 million deaths annually, underscoring its substantial impact on global health (World Health Organization, 2024).

The α-glycosidase enzyme resides in the intestinal brush border. It transforms oligosaccharides and disaccharides into monosaccharides, facilitating carbohydrate absorption and elevating blood sugar levels. α-glycosidase inhibitors can postpone carbohydrate absorption via competitive inhibition, impeding the hydrolysis of disaccharides and glucose absorption (Vichayanrat et al., 2002). The search for natural sources of α-glycosidase inhibitors including those sourced from fruits and vegetables, has attracted the attention of the scientific community. Several authors have reported the antidiabetic properties of fruit and vegetable extracts, including kiwi, lemon pulp, lemon peel, pear red onion and tomato, among others (Wu et al., 2015). In most of these cases, the antidiabetic activity was positively linked to the polyphenol content.

Regarding the date co-products under analysis, Table 3 shows that the date powder (87.62 ± 2.04 %) exhibited greater ($p < 0.05$) α-glucosidase inhibitory activity than date juice (76.34 ± 0.78%). This result was expected because date powder is richer in phenolic compounds compared to date juice (see Section 3.3), and therefore a greater α-glucosidase inhibitory activity was anticipated. Although date juice has fewer phenolic compounds (0.146 ± 0.004 mg GAE/mL), its α-glucosidase inhibitory activity is relatively high, namely 76.34 ± 0.78 %. The hypothesis is that the date fruit may also contain water-soluble non-phenolic bioactive compounds that have activity against the α-glucosidase enzyme (Khan et al., 2016; Mia et al., 2020). Similar results were reported by El Abed et al., (2017). Furthermore, a 2017 study demonstrated how various extracts from date fruits have α-glucosidase inhibitory activity, correlating the obtained results. El Abed et al. (2017) observed a strong inhibitory activity against α-glucosidase in an aqueous ethanolic extract from date fruits, which was even higher than that against α-amylase.

Furthermore, the evidence suggests that date fruit has *in vivo* antidiabetic effects. *In vivo* reports have demonstrated that short-term administration (one month intake) results in reduced blood glucose levels and increased insulin concentration through mechanisms such as an increase in the number of β-cells, stimulation of insulin secretion, and lowering of gastric emptying by the action of polyphenol (Evans et al., 2018; Mia et al., 2020).

The antidiabetic assay performed solely determines the inhibitory activity of α-glucosidase. Therefore, further *in vivo* studies are suggested to fully understand the antidiabetic capacity of both date juice and powder.

3.5. Antihypertensive activity

Hypertension is a leading risk factor for cardiovascular disease, causing widespread morbidity and mortality globally. According to WHO, one in three adults worldwide is affected by hypertension (World Health Organization, 2023). Additionally, hypertension is approximately twice as prevalent in individuals with diabetes compared to those without diabetes, making it a significant public health concern (Farida et al., 2023).

Suppressing angiotensin-I-converting enzyme (ACE) is a crucial strategy for managing hypertension, a global health crisis of epidemic scale and a significant contributor to cardiovascular disease risk (Faustino et al., 2023; World Health Organization, 2023). Lifestyle changes, including diet, have recently attracted interest due to the undesirable side effects associated with synthetic ACE-inhibitors. Consumption of fruits and vegetables, which are rich in vitamins, minerals, and bioactive compounds, has been proposed as a relevant prevention factor. Furthermore, fruits and vegetables are being investigated as potential sources of natural compounds with antihypertensive properties, which is of great interest (Yousefi et al., 2021).

Date fruits contain various bioactive compounds with potential health benefits, including antioxidant, antimicrobial, anticancer, and anti-inflammatory properties (Muñoz-Tebar et al., 2023). The antihypertensive activity of date fruits may be mainly due to the presence of flavonoids, minerals, vitamins, and fibers (Yousefi et al., 2021). Table 3 displays the ACE inhibition percentages of date juice and date powder. It is worth noting that date powder has a high ACE inhibition percentage, close to 100% (92.24 ± 0.73 %), while date juice presents an ACE inhibitory activity of 55.73 ± 4.92 %. Date fruits contain several bioactive compounds that regulate and manage hypertension, including lauric acid, linolenic acid, palmitic acid, tocopherols, β-sitosterol, and isosorbide (Obode et al., 2020). Date powder has a higher inhibition of ACE than date juice because the bioactive compounds are mainly found in the flesh, not always water soluble and therefore not transferable to date juice. However, it is important to note that date juice still has a good ACE inhibitory activity of over 50%, indicating the presence of water-soluble ACE inhibitory compounds.

Several reports have shown that date fruits have a potent antihypertensive effect due to their ability to inhibit angiotensin-converting enzyme (Al-Dashti et al., 2021; Fernández-López et al., 2022; Vayalil, 2012). This ability is mainly attributed to certain polysaccharides present in date fruits. Additionally, date fruit is rich in potassium and low in sodium, which helps to maintain electrolyte balance and further contributes to controlling blood pressure.

The valorization of co-products from the fresh date processing industry has made it possible to obtain two high-added value products with a chemical composition that is very interesting for the food industry, in an eco-efficient way and boosting the circular economy of palm-growing ecosystems. If this is complemented with their *in vitro* demonstration of important functional properties such as antioxidant, prebiotic, antihypertensive and antidiabetic activities, it will allow their application in the development of potentially healthier foods. This opens the door to their industrial processing and marketing as high added-value food ingredients. Thus, the food industry can use them for new food development and innovation projects to meet the demands of today's consumers, i.e. healthier and more sustainable foods.

4. Conclusions

The development of date by-products has made it possible to obtain two value-added food products, namely date powder and date juice. In terms of nutritional evaluation, date powder was found to be richer in nutrients and contained high levels of total dietary fiber, while date juice had high concentrations of water-soluble sugars. In this sense, date powder provided higher levels of total phenolic compounds and *in vitro* bioactivities such as antioxidant, antidiabetic and antihypertensive activities. Date juice, although poor in various nutrients, still showed activity in all the assays tested. Furthermore, in terms of prebiotic activity, the sugar-rich date juice showed a positive role for all probiotic strains tested at both 2% and 10% (w/v). Date powder also showed prebiotic potential due to its high concentration of dietary fiber. However, it is important to note that date powder in high concentrations (6% (w/v)) could inhibit probiotic growth. In this sense, this study is the first to analyse the potential bioactivities and prebiotic potential of fresh date by-products.

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Ethical statement

The authors declare that the work described has not involved experimentation on humans or animals.

Data availability

Data are available on request from the corresponding author.

CRediT authorship contribution statement

Clara Muñoz-Bas: Writing – original draft, Methodology, Formal analysis. **Rita Vedor:** Writing – original draft, Methodology, Formal analysis. **Daniela Machado:** Writing – review & editing, Methodology, Formal analysis. **Joana Cristina Barbosa:** Writing – review & editing, Methodology. **Ana Maria Gomes:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization. **José Angel Pérez-Alvarez:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Juana Fernández-Lopez:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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