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Microencapsulated saffron floral waste extracts as functional ingredients for antioxidant fortification of yogurt: Stability during the storage

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ABSTRACT

Dairy industries are constantly seeking to develop innovative ingredients through the use of food by-products, since consumers' demand for healthier foodstuffs has increased over the last years. Therefore, the development of innovative dairy products enriched with saffron floral extracts could represent a novel environmentally-friendly technological solution. The present study aimed to determine the effect of adding alginate-based microencapsulated saffron floral by-products extracts (0.5 g/100 g, 1 g/100 g), or/and saffron stigmas extracts (0.05 g/100 g, 0.1 g/100 g) to homemade yogurts. For this purpose, physical-chemical properties, antioxidant capacity, total phenolic content (TPC), microbiological analysis, color and organic acids and soluble sugars profile were determined at 0, 7, 14 and 21 days of refrigerated storage. The results showed that these novel yogurt formulations allowed proper fermentation, being the microbiological profile and physicochemical parameters not affected by saffron extracts, compared to the control. The incorporation of microencapsulated saffron floral extracts improved the functional properties in terms of TPC and antioxidant properties which remained stable over 21 days of storage. Enriched yogurts also showed a good profile of organic acids and soluble sugars, mainly lactic acid and lactose. In conclusion, saffron floral by-products-alginate beads could be potential sustainable candidates to use as functional ingredients improving the nutritional and functional value of yogurts.

1. Introduction

Currently, efforts to valorize food by-products have become of interest to the food industry, since many of them contain valuable molecules (bioactive compounds, antioxidants, fibers, etc.) which can be reused as high value-added ingredients. The function of those ingredients within the food matrix is to provide biologically health-promoting compounds, through their applications in foods for the development of novel functional products, supplements, as well as in cosmetic, pharmaceutical and human health sectors, among others (Caponio, Piga, & Poiana, 2022).

For the production of saffron spice, the current system produces a large amount of waste since only the flower stigmas are used, but the rest of the parts of the *Crocus sativus* L. plant are discarded. To obtain ≈1 kg of saffron spice, a large number of flowers (≈165,000–230,000) is needed, generating a large quantity of waste product, mostly constituted by tepals (≈78%).

However, recent studies have shown that saffron floral bio-residues contain important amounts of biologically active compounds such as flavonoids, anthocyanins, or various volatile compounds which present beneficial effects due to their potential antioxidant activities (Cerdá-Bernad, Baixinho, Fernández, & Frutos, 2022; Cerdá-Bernad, Clemente-Villalba, Valero-Cases, Pastor, & Frutos, 2022; Stelluti, Caser, Demasi, & Scariot, 2021; Zeka et al., 2020). Therefore, these functional saffron floral by-products would represent an important and promising source of natural bioactive antioxidant compounds that could be incorporated into functional foods. At the same time, the valorization of saffron floral bio-residues would lead to the minimization of their environmental impact taking advantage of a high-value biomass that is currently unexploited. In addition, it could contribute to improve the sustainability of the saffron spice production and the profitability of this industrial sector, representing also a new economic income for saffron farmers.

In order to protect valuable sensitive compounds susceptible to oxidation and degradation in the food matrix and to ensure their stability, encapsulation technologies could be an effective approach (de

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Oliveira, Fraceto, Bravo, & Polanczyk, 2021). Many polymers are employed as wall materials for encapsulation to protect the bioactive compounds, such as sodium alginate, chitosan, several gums, starch and cellulose, among others (da S. Pereira, Souza, Moraes, Fontes-Sant'Ana, & Amaral, 2021). One of the biopolymers most widely used for food applications is sodium alginate due to its properties, since shows a simple gelation with divalent cations such as Ca^{2+} and it is cheap, biocompatible, biodegradable and Generally Recognized as Safe (GRAS) (Cattelan et al., 2020; Zabot et al., 2022).

Additionally, industries, such as dairy industries, are constantly seeking to develop innovative products through the use of food by-products, since consumers' demand for healthier foodstuffs has increased over the last years (Faustino et al., 2019). Therefore, the development of innovative dairy products enriched with saffron and its floral by-products could be one of the novel technological and environmentally friendly solutions for food industries (Reguengo, Salgaço, Sivieri, & Maróstica Júnior, 2022). Yogurts are one of the most suitable traditional dairy matrices for the fortification and development of new functional products, because are among highly-consumed food in the world due to their versatility and beneficial properties (Šeregelj et al., 2021). Yogurts, fermented by *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus*, are an excellent source of proteins, essential minerals and vitamins, improving the immune system and their consumption reduces the pH in stomach, decreasing the risk of pathogen transit (O'Connell & Fox, 2001). Thus, fermented milk products could be used as vehicles to deliver nutritional and bioactive components into human diet, being fortification of yogurt a good approach to improve its functional properties (Hashemi Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015).

Therefore, this research aimed to stabilize saffron and its floral by-products extracts, rich in polyphenols, through their encapsulation in alginate beads, as well as to explore the possibility of developing fortified yogurt by including them in a traditional recipe formulation. To our knowledge, there is no available research highlighting the use of saffron stigmas and saffron floral by-products as high value-added ingredients in yogurt formulations. Therefore, the enrichment of yogurts by incorporating different concentrations of saffron and its floral extracts and the study of their physicochemical and functional properties were evaluated during storage at 4 °C (0, 7, 14 and 21 days).

2. Materials and methods

2.1. Plant material

Saffron flowers were obtained from Castilla-La Mancha (Spain), during the 2020 harvest season and cultivated following the requirements established by the Protected Designation of Origin "La Mancha Saffron". Fresh saffron floral by-products (composed by tepals, stamens and styles) were frozen in liquid nitrogen and kept at −80 °C until drying in a vacuum oven at 50 °C for 36 h to constant weight. Then, they were crushed, sieved (500 μm mesh size) and stored at −20 °C until further use. Saffron stigmas were supplied by the Spanish company Verdú Cantó Saffron Spain, S.L. being the moisture lower than 11%, crushed, sieved (500 μm mesh size) and stored at 4 °C until further use.

2.2. Preparation of extracts

Saffron floral by-products and saffron stigmas extracts were prepared using ultrapure water, in a sample/water ratio 1:50 (w/v). The extracts were shaken for 30 min in dark at 400 rpm on a magnetic stirrer at room temperature (Ovan, mod. MultiMix Heat D-MMH30E, Barcelona, Spain) and then sonicated in an ultrasonic bath (Ultrasons J.P. Selecta®, Barcelona, Spain) for 15 min and centrifuged at 11200×*g* for 10 min at 4 °C (Eppendorf Centrifuge 5804/5804R, Sigma Aldrich, St. Louis, MO, USA). Then, supernatants were filtered (0.45 μm PTFE filter, Millipore, Spain) and stored at −20 °C. All extractions were done in triplicate.

2.3. Encapsulation of extracts

Microcapsules loaded with extracts of saffron floral by-products and stigmas were prepared as described by Vinceković, Jurić, Ðermić and Topolovec-Pintarić (2017) with some modifications. Briefly, different aqueous extracts were dissolved into 1.5 g/100 mL of sodium alginate solution (Sigma Aldrich, St. Louis, MO, USA) and homogenized by mixing on a magnetic stirrer at room temperature for 60 min. Blank samples were also prepared using ultrapure water. The mixtures were dropped through a 21G x 5/8" (0.8 × 16 mm) needle into 1 mol/L $CaCl_2$ solution (Scharlau; Barcelona, Spain) under constant magnetic stirring. To allow microcapsules hardening and strengthening, they were kept at room temperature for 30 min. The microcapsules were washed three times with distilled water to remove $CaCl_2$ excess and stored at 4 °C for further analysis. Part of microcapsules were stored at −20 °C during 24 h and freeze-dried in a freeze-dryer Christ Alpha 2-4 (B. Braun Biotech International, Melsungen, Germany) for 24 h (initial temperature −25 ± 2 °C and pressure 0.220 mbar), for further use.

2.4. Characterization of encapsulated extracts

2.4.1. Total phenolic content (TPC) and encapsulation efficiency

TPC were determined using the Folin-Ciocalteu methodology as described by Cerdá-Bernad, Clemente-Villalba, et al. (2022). Briefly, 100 μL of the different extracts were mixed with 400 μL of phosphate buffer (50 mmol/L) at pH 7.8 and 2.5 mL of Folin-Ciocalteu reagent previously mixed with ultrapure water 1:10 (v/v). After 2 min, 2 mL of Na_2CO_3 (75 g/L) were added and kept at 50 °C for 10 min. The absorbance was measured at 760 nm (UV/Vis spectrophotometer T80, PG Instruments Limited, Lutterworth, UK). The results were expressed as mg Gallic Acid Equivalents (GAE) per g of sample.

The encapsulation efficiency (EE) was performed according to Pasukamonset, Kwon and Adisakwattana (2016) with slight modifications. In brief, aliquots of 50 mg of microcapsules were dissolved in 2 mL of sodium citrate (5 g/100 mL), sonicated in an ultrasonic bath for 30 min, and centrifuged at 11200×*g* for 10 min at 4 °C. The encapsulation efficiency was calculated using Equation (1):

$$EE\ (\%) = TPCe/TPCi \tag{1}$$

where TPCe is the total phenolic content encapsulated in the microcapsules, while TPCi the total phenolic content in the initial aqueous extract solution of saffron floral by-products and saffron stigmas used for the encapsulation process.

2.4.2. Swelling behavior

The equilibrium swelling ratio after freeze-drying was determined by a conventional gravimetric method. The weight of the dried sample was accurately weighed, and soaked in deionized water. The swelling behavior was measured in phosphate buffered saline (PBS) solution (pH = 7.4), at room temperature, using Equation (2):

$$\text{Swelling ratio}\ (\%) = [(m_1 - m_0)/m_0] \times 100 \tag{2}$$

where m_0 is the initial weight and m_1 is the mass swollen weight at time t. After the predetermined time points samples were extracted from the solution and weighted quickly.

2.5. Elaboration of yogurt formulations

Yogurts were made with UHT (Ultra High Temperature) whole cow milk (carbohydrates 4.6 g/100 mL, protein 3.1 g/100 mL and fat 3.6 g/100 mL) with 4% skim milk powder was used. To prepare the different formulations, encapsulated extracts were added in different concentrations, and seven lots of yogurts were then prepared: control yogurt without additional ingredients (YC), yogurt with the addition of 0.5 g/100 g (YB0.5) and 1 g/100 g (YB1) of blank encapsulated extracts, yogurt with the addition of 0.5 g/100 g (YSF0.5) and 1 g/100 g (YSF1) of saffron floral by-products encapsulated extracts, yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts (YSF0.5SS0.05), and yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts (YSF1SS0.1).

The different formulations were pasteurized at 80 °C for 30 min and cooled in an ice bath to 43 °C to aseptically inoculate the starter culture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (CHOOZIT™ MY800 LYO 5 DCU, Danisco, France), following the manufacturer's recommendations. All yogurts were prepared in triplicate. Fermentation was carried out in sterile polypropylene containers in an incubator at 43 °C until a pH of 4.6 was reached. They were then stored at 4 °C for 21 days.

2.6. Characterization of yogurt formulations

2.6.1. Physical-chemical properties: pH, acidity, total soluble solids, aw, colour, syneresis

The pH and titratable acidity (expressed as % lactic acid) were measured using an automatic titrator (TitroMatic Crison pH-Matic 23, Barcelona, Spain). For the titratable acidity (%), based on Equation (3), the titrant NaOH 1 mol/L was employed:

$$\text{Titratable acidity (\% lactic acid)} = [(V \times N \times 0.09008)/W] \times 100 \quad (3)$$

where V is the volume used of NaOH, N is the normality of titrant, 0.09008 is the milliequivalent weight for lactic acid, and W is the weight of sample used.

The determination of total soluble solids (TSS) was carried out with a digital refractometer (Hanna® HI 96801, Bedfordshire, UK) and expressed as °Brix.

The water activity (aw) of the different samples was determined using a water activity meter (Novasina AW Sprint TH 500, Pfäffikon, Switzerland) at room temperature.

The colour was measured with a Minolta CR-300 Chroma Meter (Japan) colorimeter, using L*, a*, b* scale (CIELAB system). The results were expressed as luminosity L*, a* (greenness/redness), b* (blueness/yellowness), total colour difference (ΔE), Hue angle (h°), Chroma (C*) and browning index (BI) which were calculated according to the following Equations 4, 5, 6 and 7, respectively (Milovanovic et al., 2020):

$$\Delta E = \sqrt{(L*_0 - L*_1)^2 + (a*_0 - a*_1)^2 + (b*_0 - b*_1)^2} \quad (4)$$

where $L*_0$, $a*_0$, $b*_0$ are the values of the control sample (YC) at time *t* of storage, while $L*_1$, $a*_1$ and $b*_1$ are the corresponding values for each yogurt sample at time *t* of storage.

$$h^\circ = \left(\arctan\left(\frac{b^*}{a^*}\right)\right) x \frac{180}{\pi} \quad (5)$$

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (6)$$

$$BI = \frac{[100(x - 0.31)]}{0.17}, \text{where } x = \frac{(a * +1.75L *)}{(5.645L * +a * -3.012b *)} \quad (7)$$

The syneresis of the yogurts was determined according to Lima et al. (2021), centrifuging 10 g of yogurt at 176×*g* for 20 min at 4 °C. The syneresis (%) was estimated as the weight of the supernatant released over the weight of the initial yogurt x 100.

2.6.2. Preparation of yogurt extraction

To study the antioxidant properties, total phenolic content and organic acids and soluble sugars composition, yogurt extracts were prepared following the method described by Tizghadam, Roufegari-nejad, Asefi and Jafarian Asl (2021). Ten grams of each yogurt formulation were mixed with 2.5 mL of distilled water, and 0.1 mol/L HCl was used to adjust the pH to 4. During 45 min, the mixture was kept in a water bath (45 °C) and then centrifuged at 11200×*g* for 10 min at 4 °C. The supernatants were adjusted to pH 7 using 0.1 mol/L NaOH, filtered (0.45 μm PTFE filter, Millipore, Spain) and stored at −20 °C. All extractions were done in triplicate.

2.6.3. Antioxidant properties

The antioxidant capacity was determined by using the Ferric Reducing Antioxidant Power (FRAP) method, 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method as described by Cerdá-Bernad, Clemente-Villalba, et al. (2022). Briefly, the FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) solution in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution in a volume ratio of 10:1:1, respectively. The absorbance was measured at 593 nm and Trolox (10 mmol/L) was used as standard solution (0.01–5.00 mmol/L). The ABTS radical was prepared mixing ABTS (7 mmol/L) with potassium persulfate (2.45 mmol/L), reacting for 16 h in dark at room temperature and diluting the solution with ultrapure water until its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. Trolox (10 mmol/L) was used as a reference standard (0.20–3.00 mmol/L). The DPPH radical was prepared dissolving 0.0035 g with 10 mL of methanol, reacting for 30 min in dark at room temperature. The absorbance decrease was measured at 515 nm and Trolox (10 mmol/L) was used as a reference standard (0.50–4.00 mmol/L). The antioxidant capacity results were expressed as mmol of Trolox Equivalents (TE) per 100 g of sample. All the reagents used were purchased from Sigma Aldrich (St. Louis, MO, USA).

For TPC analysis in the different formulations of yogurts, the same methodology explained in 2.4.1 was followed. The results were expressed as mg GAE per 100 g of sample.

2.6.4. Organic acids, soluble sugars and inulin content

The identification and quantification of sugars, inulin and organic acids was made by high-performance liquid chromatography using a Hewlett-Packard HPLC series 1100 equipped with a Supelcogel C-610H column (30 cm x 7.8 mm) and a Supelcoguard C-610H pre-column (5 cm x 4.6 mm) (Sigma Aldrich, St. Louis, MO, USA). The organic acids were measured at 210 nm in UV–Vis with diode array detector (DAD G1315A). For sugars, a refractive index detector (G1362A RID) was used. As a mobile phase, 0.1 g/100 mL orthophosphoric acid was used with an injection volume of 20 μl and the flow rate of 0.5 mL/min under isocratic conditions, following the methodology described by Cerdá-Bernad, Valero-Cases, Pastor, Frutos and Pérez-Llamas (2020). The concentrations were calculated through calibration curves with the standards for sugars and organic acids (Sigma Aldrich, St. Louis, MO, USA). The results were expressed as mg/g of yogurt.

2.6.5. Microbiological evaluation

Yogurts were evaluated for microbiological analysis, weighting 1 g of each yogurt formulation individually, transferred to sterile polyethylene bags with 9 mL of peptone (Oxoid, Unipath Ltd., Basingstoke, UK) and homogenized for 1 min in a stomacher at room temperature. Then, appropriate dilutions were prepared for the following bacteriological determinations: (i) *Streptococcus thermophilus*, on spread plates of M17 agar and (ii) *L. delbrueckii* subsp. *lactis* y L. *delbrueckii* subsp. *bulgaricus* on spread plates of MRS Agar, both incubated at 42 °C and 37 °C, respectively, for 48 h in an anaerobic jar with the AnaeroGen system (Oxoid S.A., Madrid, Spain). The results were expressed as Log CFU (Colony-forming unit) per gram.

Moulds and yeasts were also determined in 3M™ Petrifilm™ yeast and mould count plates (3 M, Minnesota, MN, USA). The plates were incubated under aerobic conditions at 25 °C during 48–72 h for yeasts and 72–140 h for moulds.

All these analyses were carried out on the day the yogurts were prepared (time 0) and after 7, 14, 21 days of storage at 4 °C.

2.7. Statistical analysis

Results were expressed as mean ± standard deviation. The mean comparisons were carried out using the one-way analysis of variance (one-way ANOVA) and by the Tukey's multiple range test, using SPSS version 21.0 software package (SPSS Inc., Chicago, IL). Principal Component Analysis (PCA) was conducted using XLSTAT (Microsoft Corp., Washington, DC). The significant differences were established as ($P \leq 0.05$).

3. Results and discussion

3.1. Characterization of beads containing saffron floral extracts

The encapsulation efficiency, defined by the concentration of the encapsulated material over the initial concentration used in the formulation, is one of the most critical parameters to characterize the quality of the developed microcapsules to ensure their functional properties in the final product. Then, for a successful encapsulation method, a high content and retention of the core materials must be achieved. The TPC in the encapsulated beads containing saffron floral by-products or saffron stigmas aqueous extracts were 1.243 mg GAE per g of dry beads and 1.065 mg GAE per g of dry beads, respectively. Furthermore, the EE was 55.66% in beads with saffron floral by-products and 67.55% in saffron stigmas encapsulates.

Furthermore, swelling behavior is a relevant factor of encapsulates for their practical use in food products. The swelling behavior of alginate beads as a function of time obtained is shown in Fig. 1. The results indicated that the swelling percentage of beads increased up to 3407%, 3050% and 2672 after 3 h, for blank, saffron floral by-products and saffron stigmas, respectively, presenting all the samples the same tendency regardless of the encapsulated extract. However, after 4 h, the swelling behavior could not be measured because all beads were disintegrated. This fact could be explained due to the presence of sodium ions in PBS which enter into the bead matrix and ionic exchange occurs with calcium ions, leading to increased swelling by relaxation of alginate chains. Then, the strong hydrophilicity of sodium alginate, in addition to the ion–exchange process between Na^{+} and Ca^{+2} ions, allow to a higher water-holding capacity and enhance the structural integrity of beads. Nevertheless, these ionic interactions influence in the subsequent degradation of beads, since the alginate chains begin to disintegrate and dissolve in the medium, because of the disruption of the 'egg-box' cavities (Bajpai & Tankhiwale, 2006; Hou & Wu, 2019).

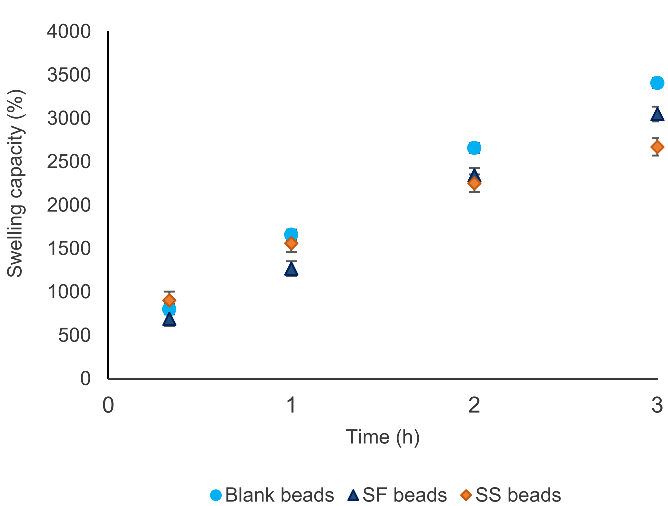

Fig. 1. Swelling behavior as a function of time of the developed beads in PBS at room temperature. The error bars represent the standard deviation (n = 3). SF: saffron floral by-products; SS: saffron stigmas.

3.2. Characterization of fortified yogurts formulations

3.2.1. Metabolism of organic acids, sugars, and physical-chemical properties of the different yogurt formulations during storage

Organic acids and soluble sugars have an effective role in terms of sensory properties and as preservatives in yogurt production. Table 1 shows organic acids, soluble sugars and inulin content in different yogurt formulations during the 21 days of refrigerated storage.

Important organic acids were present in the different yogurt formulations, as a result of the metabolism of lactic acid bacteria such as lactic acid, formic acid and propionic acid. Lactic acid was the main compound produced, and it was found in the highest concentration in all samples (1.51–2.73 mg/g of yogurt). Furthermore, in some yogurt formulations, such as YC, YB0.5, YB1 and YSF0.5, there was an increase of this organic acid over time, showing statistically significant higher amounts after 21 days compared to time 0. This increase of lactic acid was due to fermentation by LAB, using lactose to produce lactic acid. However, in YSF1, YSF0.5SS0.05 and YSF1SS0.1, the concentration of lactic acid remained stable during all the storage period, without statistically significant differences after 21 days, compared to the control yogurt (YC). Therefore, the encapsulated saffron and its floral by-products extracts did not affect this natural fermentation process in the novel developed yogurt formulations.

Formic acid was the second predominant organic acid in several yogurt formulations, such as YC, YB0.5, YB1, YSF0.5 and YSF0.5SS0.05 (0.74–1.96 mg/g of yogurt). In the fermentation process of yogurt, formic acid is produced by homofermentative metabolic pathway due to the action of starter cultures that metabolize carbohydrates present in milk (Vénica, Perotti, & Bergamini, 2014). However, in yogurt formulations with 1 g/100 g of encapsulated saffron floral by-products extracts (YSF1, YSF1SS0.1), there is a lower production of formic acid (0.35–0.76 mg/g of yogurt), which may be due to the fact that LAB, in addition to milk nutrients, could metabolize alternatively other molecules present in saffron flowers and produce different compounds. Besides, formic acid content fluctuated during the storage period in all samples, suggesting the production and consumption of this organic acid by the bacterial metabolism (da Costa, Frasao, Lima, Rodrigues, & Junior, 2016).

Propionic and citric acid were also identified in lower amounts. Citric acid, which is a predominant organic acid in milk, decreased during the storage period, and after 7 days, it was not detected in yogurt formulations, indicating its use by yogurt starter cultures. Regarding propionic acid, which was generated as the result of the metabolic activity of LAB, was not detected in some yogurt samples (YB0.5, YB1) after 21 days of storage at 4 °C, while in the rest of formulations, there was a fluctuation in its concentration, being statistically significantly lower after 21 days compared to time 0 in each sample. Therefore, these changes also suggest the generation and metabolization of propionic acid by LAB starter culture during the refrigerated storage period.

These results were in accordance to other studies that reported the presence of lactic, formic and citric acid in goat's milk yogurts, being lactic acid the most abundant one (4.75 mg/g) with varying formic and citric acid concentrations during fermentation (da Costa et al., 2016). Ndhlala, Kavaz Yüksel and Yüksel (2022) also indicated the content of lactic, citric and propionic acids in enriched yogurts with Jerusalem artichoke tubers, also changing their concentration during the storage time.

Therefore, these changes in organic acid content resulted in different pH and titratable acidity values during the storage period at 4 °C. Table 2 shows physicochemical parameters of the different yogurts incorporated with microencapsulated saffron floral extracts during the 21 days of the storage period. Statistical evaluations showed differences

Table 1
Content of soluble sugars, inulin and organic acids (mg/g) of different yogurt formulations developed during 21 days of storage at 4 °C.

	Time (days)	YC	YB0.5	YB1	YSF0.5	YSF1	YSF0.5SS0.05	YSF1SS0.1
Citric acid	0	0.17 ± 0.01^{b}	0.18 ± 0.00^{b}	0.16 ± 0.01^{b}	0.18 ± 0.00^{b}	0.18 ± 0.02^{b}	0.24 ± 0.00^{Aa}	0.18 ± 0.00^{Bb}
	7	n.d.	0.18 ± 0.03^{bc}	0.18 ± 0.00^{bc}	0.19 ± 0.00^{b}	0.17 ± 0.00^{c}	0.19 ± 0.00^{Bb}	0.30 ± 0.00^{Aa}
	14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lactic acid	0	1.76 ± 0.08^{Cb}	1.84 ± 0.02^{Cb}	1.51 ± 0.00^{Cb}	1.81 ± 0.00^{Db}	1.82 ± 0.35^{ab}	2.36 ± 0.04^{a}	1.90 ± 0.14^{Bab}
	7	2.71 ± 0.02^{Aa}	2.52 ± 0.07^{Bab}	2.13 ± 0.06^{Bde}	2.44 ± 0.01^{Abc}	2.08 ± 0.00^{e}	2.30 ± 0.01^{cd}	2.69 ± 0.07^{Aa}
	14	2.51 ± 0.11^{Aa}	2.58 ± 0.05^{Ba}	1.98 ± 0.00^{Bc}	2.07 ± 0.00^{Cbc}	1.92 ± 0.00^{c}	2.15 ± 0.00^{b}	1.72 ± 0.00^{B}
	21	2.23 ± 0.06^{Bbc}	3.19 ± 0.02^{Aa}	2.73 ± 0.07^{Aab}	2.20 ± 0.00^{Bbc}	1.96 ± 0.00^{c}	2.27 ± 0.00^{bc}	1.78 ± 0.00^{Bc}
Formic acid	0	1.48 ± 0.00^{Bb}	1.21 ± 0.03^{Bc}	0.74 ± 0.00^{Bd}	1.15 ± 0.02^{Bc}	0.40 ± 0.08^{Bf}	1.87 ± 0.03^{Aa}	0.56 ± 0.05^{e}
	7	1.96 ± 0.01^{Aa}	1.52 ± 0.03^{ABb}	0.92 ± 0.05^{Ad}	1.16 ± 0.02^{Ac}	0.35 ± 0.06^{Be}	1.48 ± 0.05^{Bb}	0.76 ± 0.10^{d}
	14	1.44 ± 0.05^{Ba}	1.37 ± 0.01^{ABa}	0.87 ± 0.03^{ABb}	0.99 ± 0.00^{Cb}	0.60 ± 0.04^{Ac}	1.46 ± 0.08^{Ba}	0.54 ± 0.04^{c}
	21	1.20 ± 0.00^{Cc}	1.62 ± 0.01^{Aa}	0.94 ± 0.03^{Ad}	0.82 ± 0.00^{De}	0.46 ± 0.01^{Bg}	1.42 ± 0.00^{Bb}	0.61 ± 0.05^{f}
Propionic acid	0	0.51 ± 0.12^{Ab}	0.58 ± 0.21^{b}	0.62 ± 0.07^{b}	0.54 ± 0.05^{ABb}	0.57 ± 0.04^{ABb}	0.62 ± 0.03^{Aab}	0.92 ± 0.06^{Aa}
	7	0.77 ± 0.18^{A}	0.78 ± 0.19	0.60 ± 0.02	0.86 ± 0.23^{A}	0.67 ± 0.09^{A}	0.55 ± 0.08^{AB}	0.87 ± 0.05^{A}
	14	0.55 ± 0.11^{Aab}	0.70 ± 0.20^{a}	0.39 ± 0.19^{b}	0.29 ± 0.00^{Bb}	0.39 ± 0.04^{BCb}	0.36 ± 0.00^{Cb}	0.43 ± 0.00^{Bab}
	21	0.14 ± 0.08^{Bb}	n.d.	n.d.	0.21 ± 0.00^{Bb}	0.31 ± 0.04^{Cab}	$0.47 \pm 0.06B^{Ca}$	0.42 ± 0.06^{Ba}
Lactose	0	12.86 ± 0.02^{Bb}	12.99 ± 0.00^{Cb}	13.51 ± 0.01^{Cb}	13.44 ± 0.00^{Bb}	16.08 ± 0.28^{Aa}	17.96 ± 0.08^{Aa}	16.39 ± 0.37^{Aa}
	7	18.15 ± 0.11^{Aa}	16.82 ± 0.28^{Bb}	17.18 ± 0.14^{Bb}	16.61 ± 0.04^{Ab}	16.32 ± 0.19^{Ab}	16.36 ± 0.09^{Bb}	16.46 ± 0.54^{Ab}
	14	17.08 ± 0.66^{Aa}	16.72 ± 0.17^{Ba}	13.53 ± 0.04^{Cb}	12.57 ± 0.01^{Cb}	13.58 ± 0.06^{Bb}	12.96 ± 0.22^{Cb}	14.10 ± 0.01^{Bb}
	21	12.79 ± 0.06^{Bb}	19.12 ± 0.88^{Aa}	18.85 ± 0.58^{Aa}	12.95 ± 0.06^{Cb}	13.40 ± 0.02^{Bb}	13.20 ± 0.03^{Cb}	13.60 ± 0.00^{Bb}
Glucose	0	0.28 ± 0.00^{B}	0.26 ± 0.01^{C}	0.30 ± 0.00^{D}	0.27 ± 0.00^{C}	0.34 ± 0.03	0.32 ± 0.00^{A}	0.36 ± 0.10^{B}
	7	0.33 ± 0.00^{Ad}	0.30 ± 0.00^{Be}	0.41 ± 0.00^{Bb}	0.31 ± 0.00^{Ade}	0.38 ± 0.00^{c}	0.31 ± 0.00^{Ade}	0.91 ± 0.01^{Aa}
	14	0.33 ± 0.00^{Ab}	0.30 ± 0.00^{Bd}	0.36 ± 0.01^{Cb}	0.28 ± 0.00^{Cd}	0.36 ± 0.00^{b}	0.29 ± 0.00^{Bd}	0.52 ± 0.00^{Ba}
	21	0.30 ± 0.00^{Bc}	0.35 ± 0.01^{Ac}	0.44 ± 0.01^{Aab}	0.29 ± 0.00^{Bc}	0.36 ± 0.00^{bc}	0.30 ± 0.00^{Bc}	0.50 ± 0.00^{Ba}
Galactose	0	1.95 ± 0.01^{Cb}	1.93 ± 0.00^{Cb}	1.67 ± 0.00^{Db}	1.98 ± 0.00^{Db}	2.08 ± 0.47^{ab}	2.76 ± 0.04^{Aa}	2.15 ± 0.27^{Bab}
	7	3.25 ± 0.05^{Aa}	2.90 ± 0.07^{Bb}	2.57 ± 0.04^{Bcd}	2.81 ± 0.02^{Ab}	2.46 ± 0.04^{d}	2.69 ± 0.00^{Abc}	3.25 ± 0.08^{Aa}
	14	3.02 ± 0.15^{aA}	2.94 ± 0.02^{Ba}	2.14 ± 0.02^{Cbc}	2.21 ± 0.00^{Cbc}	2.14 ± 0.00^{bc}	2.29 ± 0.00^{Cb}	2.52 ± 0.00^{Bc}
	21	2.42 ± 0.02^{Bb}	3.67 ± 0.23^{Aa}	3.20 ± 0.10^{Aa}	2.35 ± 0.00^{Bb}	2.17 ± 0.00^{bc}	2.39 ± 0.00^{Bb}	2.10 ± 0.00^{Bc}
Inulin	0	0.68 ± 0.00^{Bf}	1.14 ± 0.00^{Be}	1.64 ± 0.00^{Cbc}	1.17 ± 0.00^{Ade}	1.93 ± 0.30^{b}	1.54 ± 0.04^{Acd}	2.54 ± 0.05^{Ba}
	7	0.84 ± 0.02^{Ae}	1.24 ± 0.04^{ABd}	1.95 ± 0.03^{Bb}	1.17 ± 0.00^{Ad}	1.80 ± 0.03^{c}	1.24 ± 0.00^{Bd}	2.70 ± 0.04^{Aa}
	14	0.78 ± 0.03^{Ae}	1.21 ± 0.00^{ABc}	1.85 ± 0.03^{Bb}	1.09 ± 0.00^{Cd}	1.80 ± 0.00^{b}	1.16 ± 0.00^{Cc}	2.01 ± 0.00^{Ca}
	21	0.68 ± 0.00^{Bf}	1.29 ± 0.09^{Ad}	2.20 ± 0.05^{Aa}	1.15 ± 0.00^{Be}	1.80 ± 0.00^{c}	$1.21 \pm 0.01B^{Cde}$	1.97 ± 0.01^{Cb}

Means ± standard deviation in the same column followed by different uppercase letters indicate statistically significant differences at ($P \leq 0.05$) for each yogurt sample at different storage time ($n = 3$). Means ± standard deviation in the same row followed by different lowercase letters indicate statistically significant differences at ($P \leq 0.05$) between yogurt samples at the same storage time ($n = 3$); YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts;YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts; n.d.: not detected.

in the pH of each yogurt sample ($P < 0.05$) at time 0, respect to time 7, with decreasing the pH values. However, after 7 days of storage, no significant differences were observed until the end of the storage period. This pH decline is related to the lactose fermentation, leading to the production of organic acids, with subsequent lower pH values (Table 1). However, titratable acidity (%) did not show a statistical change within the different storage periods and between the different yogurt formulations, and values remained stable (0.26–0.41%). This fact may be due to the presence of some predominant organic acids formed in each storage period evaluated. Then, the continued metabolic activity of starter cultures led to deep changes in the chemical and physical attributes of yogurt formulations after the fermentation and during the refrigerated storage period, influencing on the decrease of the initial pH (4.50–4.61) to a more acidic values at day 7 (4.15–4.46). Other research observed similar results in yogurts supplemented with the maca plant (*Lepidium meyenii*) and propolis in which pH level decreased significantly from day 1–7 of storage (Korkmaz, Bilici, & Korkmaz, 2021).

As it is shown in Table 1, during the lactic fermentation process, the concentration of lactose decreased significantly over the storage time due to its conversion into lactic acid by LAB, in YSF0.5, YSF1, YSF0.5SS0.05 and YSF1SS0.1. However, lactose was not fully converted and remained stable in YC or significantly increased in blank samples (YB0.5, YB1). In addition to lactose, which was the most predominant soluble sugar in all yogurt samples (12.57–18.85 mg/g), glucose and galactose were also presented in lower concentrations (0.26–0.91 mg/g and 1.67–3.25 mg/g, respectively). Both monosaccharides were not detected in milk, but were identified in the yogurt formulations as a result of hydrolysis of lactose by the bacterial metabolic activity (Vénica et al., 2014). The concentration of both soluble monosaccharides remained stable after 21 days of storage and in some samples a slight increase was observed. Nevertheless, the concentration of galactose monosaccharide was higher than that of glucose which was very low, since galactose was not metabolized by the starter microorganisms due to the lack of essential enzymes involved in this carbohydrate metabolism.

It should be noted that these novel enriched yogurts with microencapsulated plant extracts are formulations without added sugar, since they present naturally milk sugars as lactose, as well as glucose and galactose. Besides, they are low in carbohydrates, because the most relevant sugar is lactose with levels lower than 2%. Therefore, these new yogurts with saffron flowers could be new alternatives for people with diabetes, which is the most common metabolic disease in the 21st century (Mostafai et al., 2018). In addition to the nutritional properties of yogurts, these new healthy alternatives contain saffron that could present anti-diabetic potential. Several researches reported that this natural product may exert hypoglycemic effects by different mechanisms of action (Sani et al., 2022; Yaribeygi, Zare, Butler, Barreto, & Sahebkar, 2019).

Moreover, the content of inulin as part of soluble dietary fiber was studied. The inulin concentration did not change in any of the yogurt

Table 2
Physicochemical parameters of different yogurt formulations developed during 21 days of storage at 4 °C.

	Time (days)	YC	YB0.5	YB1	YSF0.5	YSF1	YSF0.5SS0.05	YSF1SS0.1
pH	0	4.51 ± 0.06^{Ab}	4.50 ± 0.06^{Ab}	4.58 ± 0.07^{Aa}	4.59 ± 0.07^{Aa}	4.59 ± 0.07^{Aa}	4.59 ± 0.06^{Aa}	4.61 ± 0.07^{Aa}
	7	4.15 ± 0.06^{Bb}	4.14 ± 0.00^{Bb}	4.39 ± 0.01^{Ba}	4.16 ± 0.01^{Bb}	4.38 ± 0.01^{Ba}	4.18 ± 0.02^{Bb}	4.46 ± 0.03^{Ba}
	14	4.17 ± 0.08^{Bb}	4.17 ± 0.04^{Bb}	4.35 ± 0.03^{Ba}	4.20 ± 0.03^{Bb}	4.39 ± 0.01^{Ba}	4.22 ± 0.02^{Bb}	4.42 ± 0.10^{Ba}
	21	4.07 ± 0.04^{Bb}	4.02 ± 0.03^{Bb}	4.24 ± 0.01^{Ba}	4.08 ± 0.02^{Bb}	4.28 ± 0.04^{Ba}	4.06 ± 0.03^{Bb}	4.26 ± 0.07^{Ba}
TA (% Lactic acid)	0	0.30 ± 0.00^{c}	0.41 ± 0.01^{a}	0.35 ± 0.00^{b}	0.31 ± 0.00^{c}	0.30 ± 0.00^{c}	0.35 ± 0.00^{b}	0.26 ± 0.00^{d}
	7	0.31 ± 0.05	0.39 ± 0.05	0.37 ± 0.07	0.32 ± 0.07	0.34 ± 0.03	0.41 ± 0.07	0.31 ± 0.03
	14	0.33 ± 0.04	0.33 ± 0.09	0.35 ± 0.07	0.37 ± 0.07	0.32 ± 0.03	0.40 ± 0.09	0.29 ± 0.05
	21	0.31 ± 0.01	0.38 ± 0.03	0.34 ± 0.00	0.38 ± 0.04	0.37 ± 0.07	0.37 ± 0.05	0.31 ± 0.07
Water activity (aw)	0	0.89 ± 0.00	0.89 ± 0.02	0.89 ± 0.04^{B}	0.89 ± 0.05^{B}	0.89 ± 0.00	0.89 ± 0.04^{B}	0.89 ± 0.03^{B}
	7	0.90 ± 0.01^{b}	0.90 ± 0.01^{b}	0.98 ± 0.03^{Aa}	0.97 ± 0.01^{Aa}	0.90 ± 0.00^{b}	0.96 ± 0.01^{Aa}	0.97 ± 0.01^{Aa}
	14	0.89 ± 0.00	0.89 ± 0.00	0.90 ± 0.01^{B}	0.90 ± 0.02^{B}	0.89 ± 0.01	0.89 ± 0.02^{B}	0.89 ± 0.04^{B}
	21	0.89 ± 0.02	0.89 ± 0.01	0.90 ± 0.02^{B}	0.90 ± 0.01^{B}	0.89 ± 0.01	0.89 ± 0.05^{B}	0.89 ± 0.07^{B}
TSS (°Brix)	0	8.08 ± 0.11^{d}	8.38 ± 0.11^{Ad}	9.39 ± 0.13^{Ab}	8.89 ± 0.10^{Ac}	9.09 ± 0.23^{bc}	7.98 ± 0.33^{d}	9.90 ± 0.15^{Aa}
	7	8.05 ± 0.09^{c}	8.38 ± 0.11^{Abc}	8.35 ± 0.12^{Bbc}	8.01 ± 0.22^{Bc}	8.72 ± 0.05^{b}	8.35 ± 0.13^{bc}	9.26 ± 0.17^{Ba}
	14	8.05 ± 0.10^{c}	8.08 ± 0.09^{Bc}	8.38 ± 0.12^{Babc}	8.18 ± 0.06^{Bbc}	8.75 ± 0.08^{a}	8.21 ± 0.08^{bc}	8.59 ± 0.33^{Cab}
	21	8.01 ± 0.11^{b}	8.21 ± 0.10^{Ab}	8.82 ± 0.20^{Ba}	8.28 ± 0.08^{Bb}	8.85 ± 0.15^{a}	8.21 ± 0.11^{b}	9.02 ± 0.14^{Ca}
Syneresis (%)	0	17.27 ± 0.14^{Bc}	19.06 ± 0.27^{Ab}	20.93 ± 0.19^{Aa}	19.89 ± 0.18^{Aab}	20.69 ± 0.29^{Aa}	20.20 ± 0.43^{Aa}	20.76 ± 0.34^{Aa}
	7	18.83 ± 0.26^{Ab}	18.77 ± 0.26^{Ab}	19.08 ± 0.27^{Bb}	19.83 ± 0.15^{Aab}	19.58 ± 0.18^{Bab}	19.59 ± 0.13^{Aab}	20.47 ± 0.21^{Aa}
	14	19.02 ± 0.37^{A}	18.64 ± 0.26^{A}	18.52 ± 0.46^{B}	19.03 ± 0.20^{B}	18.93 ± 0.09^{C}	19.33 ± 0.23^{A}	19.32 ± 0.13^{B}
	21	18.43 ± 0.21^{Aa}	17.15 ± 0.24^{Bb}	17.80 ± 0.25^{Cab}	18.40 ± 0.26^{Ba}	18.22 ± 0.12^{Ca}	17.73 ± 0.07^{Bab}	18.71 ± 0.38^{Ba}

Means ± standard deviation in the same column followed by different uppercase letters indicate statistically significant differences at ($P \leq 0.05$) for each yogurt sample at different storage time ($n = 3$). Means ± standard deviation in the same row followed by different lowercase letters indicate statistically significant differences at ($P \leq 0.05$) between yogurt samples at the same storage time ($n = 3$); TA: titratable acidity; TSS: total soluble sugars; YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts;YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts.

formulations during storage because LAB metabolized monosaccharides as the main energy sources. Besides, at time 0 samples with saffron floral by-products extracts at 1 g/100 g presented the highest inulin content respect to the other formulations due to the rich fiber source of saffron flowers (1.93 and 2.54 mg/g, respectively). At the same time, inulin contributed to an increase in TSS, since yogurts with the highest inulin concentration presented higher TSS than control samples, such as YSF1 and YSF1SS0.1, with values around 9 °Brix (Table 2). This increase in TSS could be due to the presence of fructose in the composition of inulin.

These findings were also corroborated with a Principal Component Analysis (PCA) (Fig. 3 and Fig. S1). The PCA biplot graph (Fig. 3A) showed that the yogurt formulation YSF0.5SS0.05, at day 0, was associated to the content of citric and lactic acids, galactose and lactose. Yogurt formulations with 1 g/100 g of saffron floral by-products microencapsulated extracts, YSF1 and YSF1SS0.1, were mainly related to propionic acid and inulin content. However, after 21 days of storage (Fig. 3B), YB0.5 was the yogurt formulation associated to lactic acid, lactose and galactose, changing the composition of organic acids and soluble sugars in yogurt formulations during storage and together with the physical-chemical parameters, due to the continued metabolic activity of LAB starter cultures. Moreover, it can be also observed that yogurt formulations with 0.5 g/100 g of microencapsulated saffron floral by-products extracts YSF0.5 and YSF0.5SS0.05, had similar physicochemical characteristics and content of water-soluble compounds to the control sample YC, but they were not associated with any specific characteristic.

Regarding the physicochemical parameters, water activity and syneresis were also determined (Table 2). The water activity was not affected by either the storage period or by the yogurt formulation as interpreted statistically, since no significant differences were found between the different yogurt formulations at time 0, 14 and 21 days, nor between time 0 and 21 days of storage in each sample, being the values in the range 0.89–0.98. Similar values (0.99) were shown in previous research in yogurts incorporating preservative agents from sage and basil (Ueda et al., 2021). The syneresis ratio (serum release), a quality indicator of yogurts during storage, significantly increased in the control sample (YC) after 7 days of storage, but remained stable in YB0.5, YSF0.5 and YSF0.5SS0.05 and YSF1SS0.1 samples. After 21 days of storage, the syneresis ratio (%) significantly decreased in all samples compared to time 0, except for YC. However, at time 21 days no significant differences were found between all the yogurt formulations, with values between 17 and 18%. Initial syneresis of yogurts decreased over the time, and values were lower at the end of storage because of the pH reduction during the first 7 days, leading to a higher serum release due to the contracting effect on the casein micelle matrix (Akgün et al., 2020).

3.2.2. *Microbiological evaluation of the different yogurt formulations during storage*

In addition to carrying out a physicochemical stability evaluation to confirm that these novel yogurt formulations with microencapsulated saffron flower extracts remained with similar characteristics during storage, it was necessary to determine if, at the same time, they maintained suitable concentrations of *Lactobacillus* and *Streptococcus* starter cultures. The results of Fig. 2 showed that after 21 days of refrigerated storage, no significant differences were observed in the survival of *Lactobacillus* sp. and *Streptococcus* sp. in any of the yogurt formulations and in any of the storage periods, remaining the concentrations stable (5-6 Log CFU/g and 8-10 Log CFU/g, respectively). Therefore, yogurts fortified with saffron stigmas and saffron floral by-products were good matrices to reach a high concentration of LAB starter cultures, however, it was not possible to determine whether or not the saffron flowers can act as a prebiotic, given its encapsulated form, but they did not affect the survival of LAB. Other studies also reported that the supplementation of yogurt with chia seeds did not alter the viability of LAB, and in yogurts with the maca plant (*Lepidium meyenii*) the concentration of *Lactobacillus* remained stable during 7 days of storage (Korkmaz et al., 2021; Kowaleski et al., 2020).

Respect to the microbiological analysis of moulds and yeasts, they were not detected in any sample (<10 CFU/g) throughout the storage period, showing a good hygienic-sanitary quality during the storage for at least 21 days at 4 °C.

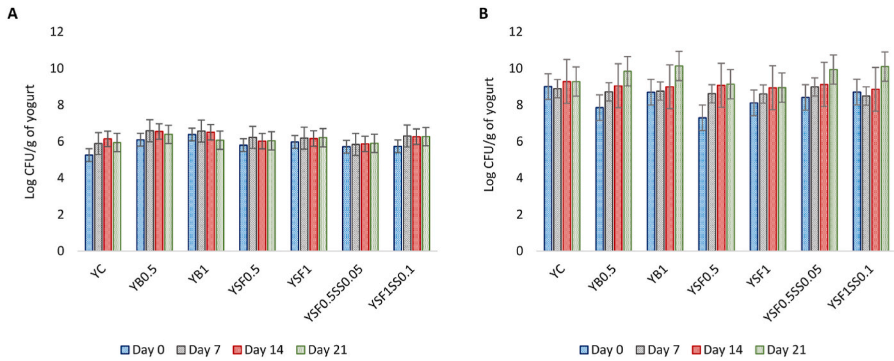

Fig. 2. **A** Viability of *Lactobacillus* sp. and **B** *Streptococcus* sp. (Log CFU/g) in different yogurt formulations during the refrigerated storage period. The error bars represent the standard deviation (n = 3). Non-labeling with different letters indicates statistically non-significant differences at (P > 0.05) for each yogurt sample at different storage time and between yogurt samples at the same storage time. YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts;YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts.

3.2.3. Colour of the different yogurt formulations during storage

Colour is also an important attribute to be evaluated in yogurts, since it is the first characteristic perceived by consumers. Table S1 shows the changes in colour parameters (L*, a*, b*, ΔE, Chroma, Hue angle and BI) of the different yogurt formulations during 21 days of storage period at 4 °C. Regarding the L* index, which indicates the brightness, no differences were found in yogurt samples with microencapsulated saffron extracts compared to YC, indicating that the addition of encapsulated saffron floral extracts did not change the lightness of yogurt samples. Conversely, yogurt formulations containing saffron stigmas extracts, YSF0.5SS0.05 and YSF1SS0.1, tended to show higher values for yellowness (b*) as expected during the storage period compared to the other yogurt formulations and this implied a change also in the chroma (C*), and thus a change in the color intensity. This fact is related to the orange-yellow color of saffron stigmas which act as natural colorant of yogurts and to the gradual release of saffron extracts from microencapsulates which protected the pigments over time (Carmona, Robert, Vergara, & Sáenz, 2021). Furthermore, due to the colorant properties of saffron, the values of total colour difference between YC and yogurt samples containing saffron stigmas (YSF0.5SS0.05 and YSF1SS0.1) were the highest at time 0, 7, 14 and 21 days of refrigerated storage (ΔE > 12), being these changes visually perceptible (Milovanovic et al., 2020). The browning index, BI, an important quality parameter and one of the most common indicators of browning in food products, significantly decreased during the storage period in all the yogurt formulations, except for the control (YC). This fact could be related to the antioxidant properties of the encapsulates that effectively reduced BI and extended the shelf life of the end product. These novel yogurt formulations with microencapsulated saffron floral by-products and/or saffron stigmas extracts showed adequate pH, acidity and sugars and acids contents, acting as preservative to improve the shelf life, avoiding microbiological or chemical spoilage and maintaining high levels of LAB starter cultures during the 21 days of storage period. Therefore, saffron and its floral by-products could be potential high value-added ingredients to develop novel dairy functional food products as their addition could contribute to maintain the quality and improve the nutritional value of yogurts.

3.2.4. Antioxidant properties and total phenolic content of the different yogurt formulations during storage

Several bioactive peptides are produced during fermentation of milk, being yogurts a rich source of antioxidant compounds with beneficial effects for health, as well as for increasing the shelf life of food products, protecting against the lipid oxidation process (Nguyen & Hwang, 2016). However, the fortification of yogurts with microencapsulated saffron floral by-products extracts or/and with saffron stigmas extracts could increase their antioxidant properties and content in bioactive compounds. Thus, the bioactivity of yogurt formulations, in terms of antioxidant activities by DPPH, ABTS and FRAP assays, and total phenolic content was studied during 21 days of storage at 4 °C. Table 3 shows the antioxidant activities and total phenolic content in the yogurt formulations developed during the 21 days of refrigerated storage.

Regarding total phenolic content, the values after 7 days of refrigerated storage, in the samples YC, YB1, YSF0.5, YSF1 and YSF1SS0.1 showed a significant increase compared to time 0. Furthermore, TPC values remained stable in all samples until the end of the storage. This fact at the beginning (time 0) may be related to the interactions of phenolic compounds with milk proteins, being maximum at pH 4.6 when proteins reached their isoelectric point. During storage, the pH decreased and thus the interactions were reduced, leading to higher TPC values after 7 days of storage. These findings were in line with those reported by Akgün et al. (2020).

Yogurt fortification with 1 g/100 g saffron floral by-products increased its functionality, since YSF1 and YSF1SS0.1 showed the highest TPC values during 21 days of storage (3.20 and 3.51 mg GAE/100 g, respectively). The incorporation of saffron floral extracts to yogurts increased their bioactivity, given that saffron floral by-products represent rich source of polyphenols, being potential ingredients with high value-added to develop new functional food products (Cerdá-Bernad, Clemente-Villalba, et al., 2022).

As it is presented in Table 3, the interaction ability of phenolic compounds with milk did not affect the free radical scavenging activity of yogurts (DPPH and ABTS assays), since values remained stable in all yogurt formulations and no significant differences were observed in terms of storage time. In addition, the results of DPPH assay revealed the significant higher antioxidant power of YSF1SS0.1 respect to the other yogurt formulations, since this yogurt sample contained the highest concentrations of saffron floral by-products and saffron stigmas microencapsulated extracts which presented a strong antioxidant capacity (Cerdá-Bernad, Clemente-Villalba, et al., 2022). FRAP values also confirmed that the incorporation of 1 g/100 g of saffron floral by-products microencapsulated extracts may be a suitable alternative for increasing the antioxidant activity of yogurts, since YSF1 and YSF1SS0.1 had significant higher ferric reducing antioxidant power (1.65 and 1.66 mmol Trolox/100 g at time 21 days, respectively) than the rest of samples, thus presenting good chelating properties to avoid lipid oxidation. These results are in accordance with those reported by Lima et al. (2021), who found an increase in the antioxidant activity of yogurts with the addition of microencapsulated fish protein hydrolysate and no differences in antioxidant activity were observed during storage.

These findings agreed with the Principal Component Analysis (PCA) biplot graph (Fig. 4) which showed that yogurt formulations incorporating 1 g/100 g of saffron floral by-products microencapsulated extracts, YSF1 and YSF1SS0.1, might be related to a greater antioxidant power such as free radical scavenging ability, revealed by DPPH and

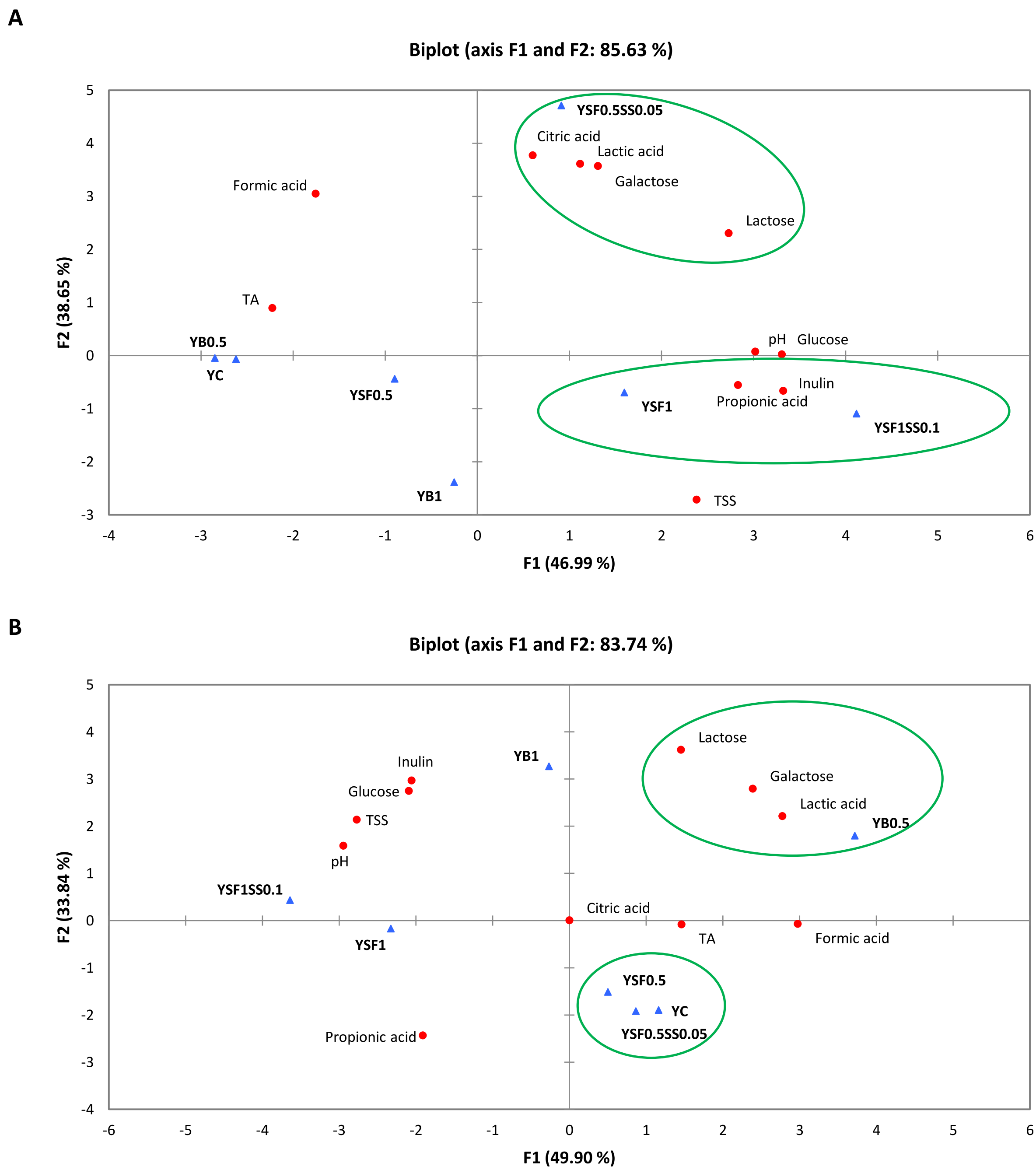

Fig. 3. PCA biplot graph at time 0 days (**A**) and 21 days (**B**) regarding physicochemical properties and organic acids and sugars content. TA: titratable acidity; TSS: total soluble sugars; YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts;YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts.

ABTS assays. In addition, it is also observed in the graph that the storage time (time 0, T0 and time 21 days of storage, T21) did not influence the antioxidant activity. Therefore, the extracts were protected by the microencapsulation from the direct contact with the food matrix environment, avoiding their oxidation and degradation, and maintaining their antioxidant activity throughout storage time. It should be noted that in other studies in which yogurts were enriched with mushroom extracts incorporated in free form, the antioxidant capacity decreased during storage due to their degradation when in contact with the yogurt matrix (Francisco et al., 2018).

Therefore, the incorporation of 1 g/100 g of microencapsulated saffron flower extracts could be a promising approach to improve the antioxidant properties and TPC of yogurts and to maintain their functionality during storage at 4 °C to exert potential beneficial effects in health after their intake.

4. Conclusions

Saffron floral by-products-alginate beads are potential candidates to be used as functional ingredients improving the bioactive potential of yogurts. The results demonstrated that alginate microencapsulation provided protection keeping the antioxidant properties of saffron

Table 3
Antioxidant properties and Total Phenolic Content (TPC) of different yogurt formulations developed during 21 days of storage at 4 °C.

	Time (days)	YC	YB0.5	YB1	YSF0.5	YSF1	YSF0.5SS0.05	YSF1SS0.1
TPC (mg GAE/100 g)	0	1.70 ± 0.08^{Bd}	1.99 ± 0.34^{Bcd}	1.71 ± 0.08^{Bd}	2.22 ± 0.18^{Bc}	2.88 ± 0.01^{Bab}	2.26 ± 0.14^{c}	3.20 ± 0.02^{Ca}
	7	2.49 ± 0.18^{Ac}	2.52 ± 0.05^{ABc}	2.75 ± 0.19^{Ac}	2.69 ± 0.23^{Ac}	3.29 ± 0.28^{Ab}	2.47 ± 0.05^{c}	4.02 ± 0.07^{Aa}
	14	2.38 ± 0.12^{Ac}	2.53 ± 0.22^{ABc}	2.45 ± 0.16^{Ac}	2.31 ± 0.16^{ABc}	3.05 ± 0.05^{ABab}	2.34 ± 0.24^{c}	3.75 ± 0.18^{ABa}
	21	2.47 ± 0.02^{Ac}	2.80 ± 0.25^{Abc}	2.60 ± 0.14^{Ac}	2.38 ± 0.02^{ABc}	3.20 ± 0.11^{ABab}	2.64 ± 0.10^{c}	3.51 ± 0.09^{Ba}
FRAP (mmol Trolox/100 g)	0	1.21 ± 0.06^{ABc}	1.65 ± 0.03^{Ab}	1.66 ± 0.09^{Ab}	1.58 ± 0.11^{b}	1.68 ± 0.09^{a}	1.56 ± 0.05^{b}	1.79 ± 0.01^{Aa}
	7	1.36 ± 0.10^{ABb}	1.46 ± 0.06^{Bb}	1.48 ± 0.07^{ABb}	1.44 ± 0.09^{b}	1.73 ± 0.07^{a}	1.50 ± 0.10^{b}	1.76 ± 0.00^{Aa}
	14	1.09 ± 0.18^{Bd}	1.24 ± 0.06^{Ccd}	1.18 ± 0.10^{Cd}	1.29 ± 0.17^{bd}	1.61 ± 0.09^{ab}	1.51 ± 0.08^{abc}	1.67 ± 0.02^{Ba}
	21	1.39 ± 0.02^{Ae}	1.49 ± 0.05^{Bbce}	1.27 ± 0.03^{BCd}	1.46 ± 0.07^{be}	1.65 ± 0.05^{a}	1.58 ± 0.02^{ac}	1.66 ± 0.03^{Ba}
ABTS (mmol Trolox/100 g)	0	0.79 ± 0.02^{ab}	0.83 ± 0.03^{ab}	0.77 ± 0.07^{b}	0.84 ± 0.04^{ab}	0.89 ± 0.05^{a}	0.84 ± 0.02^{ab}	0.89 ± 0.01^{a}
	7	0.80 ± 0.00^{b}	0.82 ± 0.04^{ab}	0.84 ± 0.02^{ab}	0.94 ± 0.09^{a}	0.94 ± 0.07^{a}	0.80 ± 0.04^{ab}	0.92 ± 0.04^{ab}
	14	0.61 ± 0.10^{c}	0.71 ± 0.10^{bc}	0.71 ± 0.11^{bc}	0.87 ± 0.02^{ab}	0.91 ± 0.06^{ab}	0.85 ± 0.00^{ab}	0.92 ± 0.02^{a}
	21	0.81 ± 0.03^{bc}	0.79 ± 0.03^{cd}	0.76 ± 0.04^{c}	0.88 ± 0.01^{ab}	0.91 ± 0.04^{a}	0.87 ± 0.03^{abd}	0.91 ± 0.00^{a}
DPPH (mmol Trolox/100 g)	0	0.27 ± 0.03^{b}	0.29 ± 0.06^{b}	0.33 ± 0.01^{ab}	0.24 ± 0.04^{b}	0.39 ± 0.08^{ab}	0.28 ± 0.09^{b}	0.48 ± 0.04^{Ba}
	7	0.26 ± 0.05^{b}	0.27 ± 0.02^{b}	0.24 ± 0.08^{b}	0.29 ± 0.03^{b}	0.32 ± 0.00^{b}	0.24 ± 0.07^{b}	0.52 ± 0.01^{Ba}
	14	0.27 ± 0.01^{c}	0.24 ± 0.01^{c}	0.28 ± 0.04^{c}	0.25 ± 0.02^{c}	0.36 ± 0.04^{b}	0.27 ± 0.03^{c}	0.66 ± 0.03^{Aa}
	21	0.24 ± 0.06^{b}	0.27 ± 0.03^{b}	0.24 ± 0.07^{b}	0.29 ± 0.06^{b}	0.32 ± 0.02^{ab}	0.29 ± 0.03^{b}	0.46 ± 0.07^{Ba}

Means ± standard deviation in the same column followed by different uppercase letters indicate statistically significant differences at ($P \leq 0.05$) for each yogurt sample at different storage time (n = 3). Means ± standard deviation in the same row followed by different lowercase letters indicate statistically significant differences at ($P \leq 0.05$) between yogurt samples at the same storage time (n = 3); YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts;YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts.

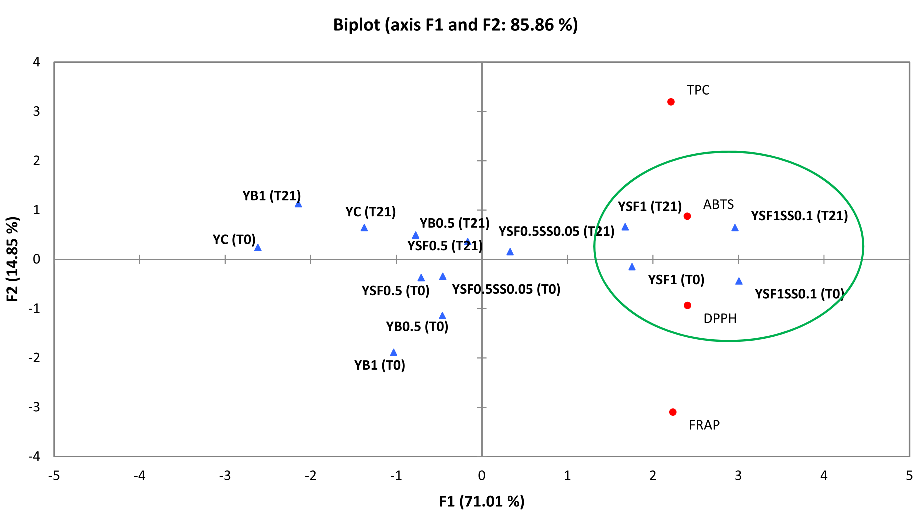

Fig. 4. PCA biplot graph at time 0 days (T0) and 21 days (T21), regarding antioxidant properties and total phenolic content (TPC). YC: control yogurt without additional ingredients; YB0.5 and YB1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of blank encapsulated extracts; YSF0.5 and YSF1: yogurts with the addition of 0.5 and 1 g/100 g, respectively, of saffron floral by-products encapsulated extracts; YSF0.5SS0.05: yogurt with the addition of 0.5 g/100 g of saffron floral by-products encapsulated extracts combined with 0.05 g/100 g of saffron stigmas encapsulated extracts; YSF1SS0.1: yogurt with the addition of 1 g/100 g of saffron floral by-products encapsulated extracts combined with 0.10 g/100 g of saffron stigmas encapsulated extracts.

flowers in the yogurt matrix over 21 days of the refrigerated storage period. Furthermore, the microbiological profile and physical-chemical parameters were not affected by the addition of saffron extracts in the novel yogurt formulations, and showed a good composition of organic acids and soluble sugars to improve the shelf life of the food product. Besides, the present study revalorizes saffron floral by-products through their application as sustainable ingredients in the production of novel enriched yogurts, improving their functionality. Hence, these novel yogurts after their ingestion can provide greater health benefits and, at the same time, the use of the saffron floral by-products extracts as ingredients in their production, taking advantage of an unexploited biomass, would lead to a reduction of the environmental impact.

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CRediT authorship contribution statement

Débora Cerdá-Bernad: Conceptualization, Methodology, Writing – original draft, preparation, Writing – review & editing. **Estefanía Valero-Cases:** Supervision. **Joaquín Julián Pastor:** Supervision, All authors have read and agreed to the published version of the manuscript. **María-José Frutos:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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