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Flavonoids, microbial load and quality parameters changes during shelf-life of fermented milk enriched with pasteurized fig purée

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ABSTRACT

Fig puree is rich in natural bioactive substances including flavonoids and can be useful to produce functional foods. This study investigated the effects of incorporating fig puree into fermented milks formulations on various physicochemical properties, and nutritional and functional compounds during 30 days of refrigerated storage. Fig puree was incorporated into cow fermented milks at 0 g 100 g^{-1} (control), 10 g 100 g^{-1}, 20 g 100 g^{-1}, 30 g 100 g^{-1} and 40 g 100 g^{-1}, respectively after fermentation. Initial pH values ranged from 4.48 to 4.84, with no significant changes observed during storage. Microbial counts remained above the acceptable threshold until 30 days in refrigeration. Color parameters indicated a decrease in lightness (L*) and hue (h) values with increasing fig puree content, while a* and b* values increased with increasing fig puree. The fig puree concentration dependently affected the texture analysis and revealed enhanced firmness, consistency, cohesiveness, and viscosity in yogurts with 40 g 100 g^{-1} of fig puree. Overall, texture stability was maintained during storage period, though slight softening was noted in some formulations. The addition of fig puree reduced syneresis compared to the control which could be attributed to the increase of pectin content. Total phenolic content and DPPH values increased with fig puree addition, with detectable anthocyanins and flavonols, predominantly quercetin-3-galactoside. Levels of these bioactive compounds increased during storage, with the highest amounts found in yogurts containing 40 g 100 g^{-1} of fig puree after 30 days of storage. These results suggest that fig puree enhances fermented milks properties and stability, offering potential health benefits due to increased bioactive compound content.

1. Introduction

Figs which is an important fruit in the Mediterranean diet are rich in fibers, minerals, sugars, organic acids, and phenolics with antioxidant capacity (Solomon et al., 2006). In this sense, fig-based products have been used historically in traditional cuisine and medicine. Over the decades, there has been an increase in the development of fig-based products (Teruel-Andreu et al., 2021) such as wine (Liu et al., 2021; Lu et al., 2021), smoothies (Cano-Lamadrid et al., 2018a, 2018b; Issa-Issa et al., 2020), fig powders (Viuda-Martos et al., 2015), biscuits (Bölek, 2021), jam (Rababah et al., 2011), and fig-milk desserts (Jahromi & Niakousari, 2018; Zare et al., 2024), among others. One of the most important qualities of figs in the development of new food products is focus to provide them with an attractive colour, enhance their taste and techno-functional properties, and beneficial qualities (Backes et al., 2018, 2020).

With regard to the environmental footprint of fig cultivation, the aim is to avoid wastage. Therefore, it is necessary to develop products with non-commercial figs (food loss by FAO definition) for different reasons such as undersized figs, over-ripening and harvest-related damage. Apart from “food loss”, the functional properties of fig by-products during processing (food loss by FAO definition), have been widely studied and stands out for its content of fibre, organic acids, sugars, and anthocyanins (Teruel-Andreu et al., 2023; Wojdyło et al., 2016), being a good change to develop fig-based food products. Therefore, the development of new fig-based products could not only be an increase of their functional and techno-functional value, but could also represent an opportunity to mitigate "food loss" (Teruel-Andreu et al., 2021).

Fermented milks have been defined as an accurate food matrix to incorporate fruit by-products (FAO, 2007) such as citrus fibers (Sendra

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Table 1
pH, microbial load (estimated *Lactobacillus* and *Lactococcus*) and CIE*Lab* color parameters of developed fermented milks at T0 (24 h) and T30 (30 days, refrigerated storage).

	pH	Lactobacillus (log UFC g^{-1})	Lactococcus (log UFC g^{-1})	L*	a*	b*	C	h
ANOVA [a,b]	***	NS	***	***	***	***	***	***
CTRL T0	4.48 ± 0.03 b	6.53 ± 0.27	9.00 ± 0.06 a	78.6 ± 0.6 a	−1.85 ± 0.03 e	5.05 ± 0.03 d	5.37 ± 0.02 d	110 ± 12 a
F 10 T0	4.49 ± 0.01 b	6.53 ± 0.18	8.94 ± 0.07 ab	70.5 ± 0.1 b	1.65 ± 0.08 d	5.41 ± 0.08 cd	5.66 ± 0.04 cd	73.0 ± 6.1 b
F 20 T0	4.70 ± 0.18 ab	6.45 ± 0.18	8.71 ± 0.12 b	64.9 ± 1.2 c	2.65 ± 0.05 c	5.82 ± 0.08 bc	6.39 ± 0.03 bc	65.6 ± 4.2 c
F 30 T0	4.84 ± 0.18 a	6.69 ± 0.10	8.73 ± 0.01 ab	59.3 ± 1.7 d	3.34 ± 0.08 b	6.04 ± 0.21 b	6.91 ± 0.04 ab	61.0 ± 6.0 d
F 40 T0	4.81 ± 0.01 a	6.43 ± 0.04	8.90 ± 0.01 ab	56.1 ± 0.3 e	4.06 ± 0.21 a	6.54 ± 0.33 a	7.70 ± 0.02 a	58.2 ± 6.0 e
CTRL T30	4.51 ± 0.04 b	6.50 ± 0.03	9.01 ± 0.05 a	78.8 ± 0.6 a	−1.85 ± 0.21 e	4.67 ± 0.15 e	5.02 ± 0.03 de	112 ± 13 a
F 10 T30	4.53 ± 0.02 b	6.49 ± 0.04	8.89 ± 0.04 ab	70.6 ± 0.7 b	1.70 ± 0.17 d	4.93 ± 0.11 e	5.21 ± 0.05 d	71.0 ± 7.0 b
F 20 T30	4.68 ± 0.01 ab	6.58 ± 0.16	8.77 ± 0.04 ab	64.5 ± 0.3 c	2.74 ± 0.04 c	5.43 ± 0.03 cd	6.09 ± 0.03 c	63.2 ± 4.0 cd
F 30 T30	4.88 ± 0.03 a	6.54 ± 0.15	8.64 ± 0.03 b	58.6 ± 0.6 d	3.41 ± 0.08 b	5.73 ± 0.18 cd	6.67 ± 0.21 b	59.2 ± 3.1 de
F 40 T30	4.86 ± 0.02 a	6.59 ± 0.16	8.61 ± 0.04 b	55.9 ± 0.8 e	4.05 ± 0.02 a	6.33 ± 0.07 ab	7.51 ± 0.03 a	57.4 ± 7.2 e

*, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[a] NS = not significant ($p < 0.05$).
[b] Values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test.

et al., 2008), date syrup (Shahein et al., 2022), pomegranate peel (El-Said et al., 2014), among others. Apart from knowing the functional and techno-functional properties during food processing and the consumer acceptance of developed product, it is also important to understand the behaviour of fermented milk enriched with fruit by-products during refrigerated shelf-life.

One of the key parameters to consider is the stability of the gel, which may fluctuate under refrigeration (Saint-Eve et al., 2008). Changes in lactic acid bacterial load during refrigeration could be changed affecting the functional properties (probiotic levels, stability of flavonoids) (Jakobek & Matić, 2019) and the main quality parameters during shelf-life (Gris et al., 2007).

Taking all the aforementioned factors into consideration, the objective of the study was to investigate the changes of functional aspects (microbial load and flavonols) as well as techno-functional properties (colour, texture, organic acids, and sugars) of fermented milks enriched with different concentrations of pasteurized "Colar" fig puree (came from fig by-products) during refrigerated storage.

2. Materials and methods

2.1. Pasteurized fig puree and fermented milk manufacture

To prepare the fig puree, we utilized Colar variety figs sourced from Albatera farms in Alicante, Spain, harvested during the 2021 season and stored frozen until processing. Figs were harvested in the fields, only clean, whole fruits where collected. Figs were daily delivered to a fruit processing plant where they were classified according to size, ripening stage and postharvest damage. The discarded fruits were either damaged, under commercial size, or not in the proper ripening stage. Such fruits were stored under refrigeration conditions till the following morning when we collected them from the fruit processing plant. Once in our facilities figs were frozen at −20 °C until higienization and use. Following this procedure, figs were frozen within less than 48 h after harvesting. From now on, the figs underwent a disinfection process by immersing them in a solution of 200 mg L^{-1} of peracetic acid (Citrocide® PC, Citrosol, Valencia, Spain) at 15 °C for 10 min, followed by rinsing with running water at 15 °C for 5 min (5 L s^{-1}). The puree was prepared using a Thermomix®, starting with fruit crushing (1-min, speed 5; 1-min, speed 7), followed by thermal treatment (100 °C, 20 min). Pasteurized fig purée was distributed in sterile cups and cooled in a water bath with ice to decrease the temperature below 4 °C as fast as possible to enhance the efficiency of the pasteurization treatment. From previous experiments with fruit added fermented milks authors concluded that the best procedure to reduce anthocyanins decay due to microbial degradation is to add the fruit after fermentation (Cano-Lamadrid et al., 2017). Additionally, and to better yogurt texture development yogurts were stored under refrigeration overnight. This is the reason why yogurts as well as purees were cold stored overnight (24 h) to mix them when cooled. It is known that contamination by molds because of damage to figs could reduce quality and increase the risk of aflatoxin and ochratoxin. Therefore, triplicated samples from two harvest days were sent to an external laboratory for mycotoxins analysis by LC MS-MS and the following results were obtained. All tested mycotoxins were under the detection limits of the technique (Aflatoxin B1 <1.0 μg kg^{-1}; Aflatoxin B2 <1.0 μg kg^{-1}; Aflatoxia G1 <1.0 μg kg^{-1}; Aflatoxin G2 <1.0 μg kg^{-1}; Desoxinivalenol <50 μg kg^{-1}; Fumonisins sum (B1 + B2) <20 μg kg^{-1}; Ocratoxin-A <1.0 μg kg^{-1}; Patulin <10 μg kg^{-1}; and, Zearalenone <10 μg kg^{-1}) and analyzed fig by-products fulfilled the requirements of EU regulations as indicated below (Maximum Residue Level MRL sumatory of Aflatoxins 10 μg kg^{-1} and Ocratoxin A 8.0 μg kg^{-1} by R396/2005 of the European Parliament and of the Council of February 23, 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending; Council Directive 91/414/EEC. Current consolidated version: May 11, 2024).

For producing fermented milk were using UHT whole cow's milk (3.6 g 100 mL^{-1} fat content, Hacendado, Spain) and lyophilized concentrated lactic ferment containing *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp. *lactis, and Lactobacillus delbrueckii* ssp. *bulgaricus* (CHOOZITTM MY800 LYO 5 DCU, Rhodia Food-Danisco A/S, Sassenage, France). Fermented milk was made following previous studies (Cano-Lamadrid et al., 2017; Jiménez-Redondo et al., 2022; Muelas et al., 2022). with some modifications. To facilitate dosage according to manufacturer instructions the lyophilized culture was poured into 20 mL of sterile peptone water (Merck KGa, SigmaAldrich, United States) and kept in a water bath at 43 °C for 20 min to hydrate. One litre capacity Pyrex bottles were sterilised, and UHT milk was added under hygienic conditions (laminar flow cabinet) adding milk volumes according to the formulation to be developed (ranging from 1 L for control and 600 mL for those formulations to have 40 g 100 g^{-1} fig puree). Rehydrated culture dosage was 1000 μL per litre of milk. Once the starter culture was inoculated into the bottles, they were manually shaken for 1 min and incubated at 43 °C until they reached a pH of 4.6. Once this pH was reached, they were refrigerated for 24 h before being mixed and shaked with the fig puree. Finally, a determined amount of

pasteurized puree (0, 10, 20, 30, and 40 g 100 g^{-1}) was weighed and added to each fermented milk bottle, which were manually shaken until obtaining a homogeneous drinking fermented milk. It was dispensed into sterile containers and kept refrigerated for 24 h at 4 °C. Finally, the fermented milks were kept refrigerated for analysis at two sampling times: i) T0 (24 h of refrigerated storage) and ii) T30 (30 days of refrigerated storage).

2.2. Microbial load and techno-functional properties analysis

MRS agar was utilized for enumerating *Lactobacilli* counts (LAB) at 37 °C under microaerophilic conditions for 48 h, while M17 agar was employed for *Lactococci* counts (LAC) at 30 °C under aerobic conditions for 48 h. Rose Bengal Agar was employed for detecting molds and yeasts at 26 °C under aerobic conditions for 72 h. The color characteristics of fermented milks were investigated within the CIELab* color space, evaluating parameters such as lightness (L*), redness (a*, green-red coordinate), and yellowness (b*, blue-yellow coordinate). Color determinations were conducted at 12 ± 2 °C using a Minolta CM-2002 spectrophotometer, equipped with a liquid accessory CR-A70, with illuminant D65 and an observer of 10°. The equipment underwent daily calibration with the provided white plate by Minolta. pH was measured in all batches to monitor the fermentation process and to ensure that the pH reached the value of 4.6 as indicated above. Additionally, the pH was measured in the fortified fermented milks after incorporation of the fixed g 100 g^{-1} of pasteurized fig puree at time 0 and at time 30 (these values are shown in Table 1). Three replicates were conducted for pH and microbiology, while nine replicates were performed for color assessment. A penetration test was executed using a Texture Analyser TA-XT2 with a 5 kg load cell. Constant speed penetration tests were conducted directly on cylindrical containers (4.5 cm diameter, 4 cm height). All instrumental texture analyses were carried out at 8 °C after removing spontaneous syneresis. This test is 'destructive' as it does not allow structure recovery. A cylindrical probe with a diameter of 10 mm (P-10) was inserted 15 mm into the samples at a speed of 1 mm s^{-1}. Triplicate measures were taken for each yogurt. Gel stability was visually assessed post-incubation (spontaneous syneresis) and determined by quantifying the volume of whey removed from the curd after centrifugation (syneresis). A Hewlett Packard HP-1100 instrument (Woldbronn, Germany) coupled with two detectors: DAD G1315A (set at 210 nm) and RID G-1362 A was used for analysing organic acids and sugars in fermented milks (Jiménez-Redondo et al., 2022). Chromatographic analysis was performed in isocratic gradient with a flow of 0.5 mL/min and a mobile phase consisting of ultrapure water acidified with 0.1 % phosphoric acid. The column used was a Supelcogel C-610H, 30 cm × 7.8 mm (Supelco Park, Bellefonte, PA, USA). Sugars were detected with a refractive index detector and lactic acid with a diode array (DAD) at a wavelength of 210 nm. The quantification was carried out using external calibration curves prepared with pure standards of sugars and lactic acid (Merck KGaA, Darmstadt, Germany).

2.3. Total polyphenolic content (TPC), antioxidant capacity and flavonoids identification and quantification

0.5 g of fresh sample were used and 4 mL of extracting solution methanol/water/formic acid (80:19.9:0.1, v/v) was added for phenolic compounds extraction. The tubes were shaken in an orbital bath (Unitronic 320 OR, Selecta, Barcelona, Spain) with ice for 10 min at 250×*g* and 4 °C sonicated (Model 3000512, Selecta, Barcelona, Spain) for 10 min and centrifugated at 4000×*g* for 10 min at 4 °C (Sigma 3−18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany). Supernatants collected after centrifugation are re-extracted additionally 2 times and quantified in triplicate using the Folin−Ciocalteu reagent, with results expressed as mg gallic acid equivalent per gram of fresh weight (fw). These extracts were used for antioxidant capacity (DPPH) as per the method by (Brand-Williams et al., 1995), with results expressed as mmol Trolox equivalents (TE) per gram of fresh weight (fw). For flavonoids determination, extracts were filtered through a 0.45 μm pore size membrane filter before injection in the LC–MS/MS system (LC-MS/MS 8050, Shimadzu, Kyoto, Japan). The method of LC–MS/MS analysis was performed according to the procedure described by (Uysal et al., 2023) with a slight modification. The column temperature (Mediterranea SEA 18, 10 mm L x 0.21 mm i.d., 2.2 μm, Teknokroma, Barcelona, Spain) was set at 50 °C. The mobile phase consisted of two solvents: (i) Solvent A, water/formic acid (99.9:0.1, v/v) and (ii) Solvent B, acetonitrile/formic acid (99.9:0.1, v/v). Anthocyanin compounds were eluted as following conditions: 0.4 mL/min fow rate and 30 °C, isocratic conditions for 1 min with 99 % A, from 1 to 15 min linear gradient of 1–40 % acetonitrile with 0.1 g 100 mL^{-1} formic acid (B), solvent B was increased to 100 %, between 15 and 23 min, then returned to initial conditions of 99 % A in 2 min, and isocratic conditions with 99 % of 1 g 100 mL^{-1} aqueous formic acid for 5 min followed by washing and reconditioning the column. The sample volume injected was 10 μL. The sample volume injected was 10 μL. The ultraviolet visible (UV–visible) spectra were scanned from 200 to 600 nm for all peaks. The analysis was performed in triplicate for each sample. The identification was acquired using authentic standards and comparing the retention times and UV–visible spectra with those found in the literature. The characterization of the single components was carried out via the retention time and the accurate molecular masses. The PDA spectra were measured over the wavelength range of 200–600 nm. The runs were monitored at the following m/z: cyanidin 3,5-diglucoside at 611.10, quercetin-3-galactoside at 465.00, and quercetin-3-glucoside at 463.25. Retention times (Rt) and spectra were compared with pure standards.

2.4. Statistical analysis

Statistical analysis and comparison among means were carried out using the statistical package SPSS 24.0 (IBM SPSS Statist cs, Chicago, IL, USA). One–way ANOVA test was carried out, followed by Tukey's test (95 % confidence level). Principal component analysis (PCA regression map) was conducted to project the samples depending on the techno-functional parameters and microbial load.

3. Results and discussion

3.1. Microbial load and metabolic products

Table 1 shows the pH values, observing statistically differences among formulations, but no difference was reported between sampling time (T0-30). The initial pH values of the control, F10, F20, F30 and F40 fermented milks samples were 4.48, 4.49, 4.70, 4.84 and 4.81, respectively. As expected, the addition of pasteurized fig puree increased the pH of the final fermented milks. The range of values of fig fruits reported by other authors is above the value of the fermented milk (between 5.2 and 6) (Pereira et al., 2017). Our results are in accordance with previous studies; Feng et al. (2019) observed no significant differences in the pH values between jujube juice enriched yogurt formulations during monitored storage period. It could be said that the main reason is the zero or low production of lactic acid by lactic acid bacteria during refrigeration conditions.

As to microbial load, different behaviour was observed between *Lactobacillus* and *Lactococcus*. In terms of formulation, although no statistically significant differences were reported for *Lactobacillus*, statistically significant variations were observed for *Lactococcus*. It is important to highlight that no differences in *Lactobacillus* (above 6.5 Log LAB CFU g^{-1}) and *Lactococcus* (above 8.6 Log LAB CFU g^{-1}) were noted between sampling time (T0-30). The microbial load was adequate, being above 6 Log CFU/g the level established by the International Recommendations for Fermented Milks (FAO, 2003). The observed trend aligns with the findings reported in the pH section. Contrary, previous studies in which fermented milks was enriched with different fruits reported a reduction

Table 2
Texture parameters and gel stability of developed fermented milks at T0 (24 h) and T30 (30 days, refrigerated storage).

	Firmness (g)	Consistency (g·seg)	Cohesiveness (g)	Viscosity index (g·seg)	Syneresis
ANOVA [a,b]	**	**	**	**	**
CTRL T0	15.3 ± 3.21 c	378 ± 72 c	−11.8 ± 2.3 a	−5.54 ± 1.01 c	71.4 ± 0.5 a
F 10 T0	15.7 ± 2.48 c	404 ± 87 bc	−10.9 ± 1.6 a	−2.44 ± 0.94 a	66.0 ± 2.3 b
F 20 T0	16.0 ± 0.85 bc	396 ± 30 bc	−12.4 ± 0.7 a	−5.26 ± 0.58 bc	59.3 ± 0.7 c
F 30 T0	17.8 ± 1.74 b	449 ± 50 b	−14.0 ± 1.5 bc	−11.3 ± 1.78 d	56.5 ± 0.4 d
F 40 T0	19.7 ± 1.26 a	512 ± 33 a	−16.4 ± 0.6 c	−22.8 ± 3.26 e	56.7 ± 0.6 d
CTRL T30	14.5 ± 1.24 d	351 ± 21 d	−11.1 ± 0.4 a	−4.15 ± 0.52 b	73.8 ± 0.4 a
F 10 T30	14.6 ± 0.98 cd	360 ± 17 cd	−11.6 ± 0.2 a	−2.42 ± 0.41 a	65.8 ± 0.7 b
F 20 T30	15.8 ± 0.24 c	389 ± 11 c	−11.9 ± 0.9 a	−2.85 ± 0.33 a	61.5 ± 0.2 bc
F 30 T30	17.3 ± 0.11 b	437 ± 57 b	−13.1 ± 1.2 ab	−9.08 ± 1.11 d	60.9 ± 0.6 cd
F 40 T30	19.3 ± 1.10 a	498 ± 49 b	−15.8 ± 1.1 c	−20.8 ± 2.10 e	56.1 ± 0.7 d

*, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[a] NS = not significant ($p < 0.05$).
[b] Values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test.

in the microbial load after 20–30 days of refrigerated storage (Almusallam et al., 2021; Feng et al., 2019; Mahmoudi et al., 2021; Ning et al., 2021; Silva et al., 2022; Taheur et al., 2023). This could be due to post-acidification throughout refrigerated storage in fermented milks. According to the results obtained, it can be concluded that fig by-products could be suitable to the development of fermented products with lactic acid bacteria, maintaining their viability and ensuring probiotic effects.

Significant changes in organic acids and sugars changes were observed as an effect of formulation and sampling time. Figs from the "Colar" variety employed in developed fermented milks was rich in sugars was reported by Teruel-Andreu et al. (2023) had values for glucose and fructose in fresh pulp of "Colar" figs were 379 g kg^{-1} and 364 g kg^{-1} dried weight, respectively. Similarly, the content of these sugars in pasteurized fig puree was quantified, being 155 g kg^{-1} and 156 g kg^{-1}, respectively. As expected, the addition of fig puree resulted in increased glucose and fructose content, with the highest levels detected in F40, followed by F30, F20, and F10, compared to the CTRL. Galactose was also detected in CTRL sample, but it was not possible to estimate the galactose content in the fermented milk enriched with fig puree due to the large size of area of fructose and the proximity of their retention times (12.86 min for galactose and 13.05 min for fructose). Changes in the content of lactic acid was observed between sampling time, being in all samples higher at T30 given lactic acid metabolism during refrigerated storage.

3.2. CIELab coordinates

As to CIELab* coordinates, significantly differences ($p < 0.05$; Table 1) was found among formulations and between sampling times. Compared with the control, the a* and b* and C values of fermented milks developed increased with increasing amounts of puree fig, whereas their L* and h values decreased. Generally, incorporation of FP in fermented milks significantly decreased L* values compared with the control, indicating that decreased the lightness and a light pink colour formation. After 30 days of storage, the color of FP fermented milks samples kept constant for L* and a* coordinates, although the b* and C values showed a decreasing tendency in all formulations except the C value for formulation F40 at T30, where the result remains constant. For h, the results obtained among the formulations after 30 days of storage were variable, with formulations F20 at T30 and F30 at T30 decreasing, while the rest of the formulations remained constant. Our results were similar to these authors that reported a decreasing trend during storage, especially for the luminosity (L*) in enriched yogurt with edible anthocyanin-rich plant materials such as mulberry pomace (Du et al., 2021, 2023), pomegranate (Cano-Lamadrid et al., 2017), cherry (Sánchez-Bravo et al., 2018), grape (Silva et al., 2022). The decreasing trend during cold storage of certain color parameters is mostly caused by the degradation of the bound pigments during storage (Du et al., 2023). In addition, the pH of the medium has been reported as the main cause of anthocyanin color changes (Cheynier, 2012), but in our study, no changes in pH were detected during the storage period. Other authors have reported that there was no statistically significant effect of storage time on the color of natural plain yogurts (Jakubowska & Karamucki, 2019).

3.3. Texture parameters and gel stability

Table 2 shows texture parameters and syneresis of developed fermented milks. Statistically differences among formulations and between sampling time (T0-T30) was reported in this study. Taking the formulation into account, the highest values of firmness (19.74 g), and consistency (511.82 g seg) were observed when 40 g 100 g^{-1} of fig puree was added (F40), followed by the other percentages in both sampling times (T0 and T30). The lowest values of cohesiveness (−16.42 g) and viscosity index (−22.83 g seg) were observed when 40 g 100 g^{-1} of fig puree was incorporated (F40), followed by the other percentages. When considering the sampling time, it is important to note that formulations with 30 g 100 g^{-1} and 40 g 100 g^{-1} fig puree showed no statistically significant changes in firmness values between T0 and T30. In addition, statistically differences of consistency were observed in CTRL, F10 and F20 between sampling time, while consistency was maintained in the rest of the formulations. However, the cohesiveness values remained constant, while there was fluctuation in viscosity results with lower results for CTRL and F20 respect initial time, but constant for the rest of formulations. Overall, F30 and F40 remained constant during the 30 days of storage, with only changes slightly in consistency and cohesiveness for F40 and F30, respectively. Our results indicate that incorporating fig puree enhanced certain techno-functional parameters, particularly when 30 g 100 g^{-1} and 40 g 100 g^{-1} of fig puree were added. This may be due to figs, especially the peel, have a high content of pectin (Gharibzahedi et al., 2019). Pectin or plant polysaccharide has proven effective protein aggregation and reduces serum separation in yogurt (Foley & Mulcahy, 1989). Additionally, its use increases viscosity in acidic milk gels and contributes to textural stabilization in stirred yogurt (Amice-Quemeneur et al., 1995). Previous studies reported an improvement in texture and increased viscosity in yogurts fortified with plant material high in pectin such as lemon peel powder (Rahman et al., 2024), apple pomace (Wang et al., 2019), and orange fibre (Kieserling et al., 2019).

In terms of syneresis, statistically significant differences were observed among formulations; as the percentage of fig puree increases, the syneresis of fermented milk decreases. Also, statistical difference was reported between sampling time (T0-T30). A low incidence of syneresis suggests an enhanced capacity to retain water, which usually correlates with higher gel strength (Huang et al., 2021). Fermented milks with 20 g 100 g^{-1} and 30 g 100 g^{-1} of FP at T30 showed higher syneresis values compared to the ones at T0. The increase in g 100 g^{-1}

Table 3
Organic acid and sugars (g kg^{-1}), total phenols content (TPC) and antioxidant capacity (DPPH assay) of developed fermented milks at T0 (24 h) and T30 (30 days, refrigerated storage).

	Malic acid	Lactic acid	Lactose	Galactose	Glucose	Fructose	TPC (g GAE kg^{-1})	DPPH (mmol Trolox kg^{-1})
ANOVA [a,b]	*	*	**	**	**	**	**	*
CTRL T0	nd	51.3 ± 1.1 ab	237 ± 12 a	68 ± 2 a	nd	nd	36.3 ± 2.2 c	13.1 ± 0.7 b
F 10 T0	1.0 ± 0.1 b	48.0 ± 1.0 bc	221 ± 6 ab	nd	17.2 ± 1.0 de	26.3 ± 1.1 d	45.6 ± 9.8 bc	13.9 ± 0.9 ab
F 20 T0	2.0 ± 0.1 b	43.8 ± 3.1 cd	204 ± 3 b	nd	32.7 ± 1.1 c	39.3 ± 0.9 c	50.1 ± 8.8 abc	14.6 ± 0.3 ab
F 30 T0	3.0 ± 0.1 ab	42.2 ± 5.2 d	189 ± 7 c	nd	46.7 ± 0.9 b	52.7 ± 2.1 b	57.1 ± 6.8 ab	14.3 ± 1.0 ab
F 40 T0	5.0 ± 0.1 a	42.8 ± 2.3 d	173 ± 6 c	nd	61.7 ± 1.0 a	66.7 ± 1.3 a	64.4 ± 3.6 a	15.3 ± 0.7 a
CTRL T30	nd	60.7 ± 2.0 a	248 ± 5 a	51 ± 4 b	nd	nd	32.1 ± 1.9 d	12.6 ± 0.5 c
F 10 T30	1.0 ± 0.1 b	60.1 ± 1.9 a	235 ± 8 a	nd	13.7 ± 0.8 e	21.3 ± 0.9 e	45.2 ± 2.5 bc	12.6 ± 0.2 c
F 20 T30	2.5 ± 0.1 b	52.7 ± 1.8 ab	216 ± 10 ab	nd	34.7 ± 1.0 c	32.5 ± 1.1 cd	51.3 ± 3.7 abc	13.0 ± 0.8 b
F 30 T30	3.3 ± 0.1 ab	49.0 ± 1.7 b	192 ± 4 c	nd	48.0 ± 1.1 b	43.7 ± 1.4 cd	54.8 ± 2.5 abc	13.3 ± 0.6 b
F 40 T30	4.1 ± 0.1 a	47.7 ± 2.2 bc	162 ± 6 c	nd	60.3 ± 1.7 a	63.8 ± 1.3 ab	65.5 ± 2.7 a	13.2 ± 0.7 ab

*, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[a] NS = not significant ($p < 0.05$).
[b] Values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test.

syneresis during storage is in agreement with other studies (Ramirez-Santiago et al., 2010) but Sánchez et al. (2020) reported that yogurt would be less susceptible to syneresis during storage if its water retention capacity is improved.

As mentioned earlier, the pectin found in the fig puree may contribute to enhanced gel stability, resulting in reduced syneresis. Other aspects in which the fig puree may have influenced the texture include increase the solids and increase the phenolic compounds. It is common to increase the solids of commercial yogurts to improve the texture of the final yogurt (Jaster et al., 2018) and the phenolic compounds in FP could interact with caseins to form soluble complexes and further enhance the gel strength of set yogurts (Kumar & Mishra, 2003).

On the other hand, it is worth mentioning that figs and fig latex contain several proteases with high specificity for casein as a substrate and so able to coagulate milk. Most of them could be inactivated at temperatures above 60 °C which is much lower than the used in the present study to pasteurize fig pure. Overall, authors expect most textural changes to be due to fruit polysaccharides but to proteolytic changes due to fig enzymes that were mostly inactivated. Crude fig protease extract has been reported to have stable proteolytic activity in a pH range of 6.5–9.0 (optimal at pH 7–8) but lose activity, at pH 2–3. The proteolytic activity of the fig extract is stable up to 60 °C but declines at higher temperatures (Kim et al., 2011). The main protease in fig is ficin (Cysteine Proteases), and when isolated maintains activity up to 72 °C, but the stability declines at higher temperatures, and it is active within a pH range of 6.5–8.5, with maximum activity observed at pH 7.0 (Devaraj et al., 2008). Ficin Isoforms (A, B, C, D1, D2) from fig latex exhibit varying degrees of thermal stability, however, all of them are prone to autolysis at high temperatures (Zare et al., 2013).

3.4. Functionality

The total phenolic content (TPC) and radical scavenging rate (DPPH) are presented in Table 3. The developed fermented milks exhibited a rising trend in both total phenolic content (TPC) and DPPH radical scavenging activity with increasing amounts of added fig puree. This finding was attributed to the abundant polyphenols and high antioxidation potential of figs; thus, recent studies have highlighted the high antioxidant potential of figs (Teruel-Andreu et al., 2021, 2023; Wojdyło et al., 2016). After 30 days of storage, the TPC of FP fermented milks samples kept constant except for CTRL and F30 formulations that decreases slightly. While, for DPPH all formulations showed a decreasing tendency, obtaining values between 1 and 1.2 times lower. Muniandy et al. (2016) studied the influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage and indicated that TPC of all tea yogurts analyzed decrease after 21 days storage. In contrast, these authors Du et al. (2021) indicated the content TPC increase of mulberry pomace-fortified stirred yogurts during cold storage at 4 °C. These differences in results may be due to the interactions between the compounds of the added plant material and the components of the milk, which affect the bioavailability or degradation of the antioxidant compounds.

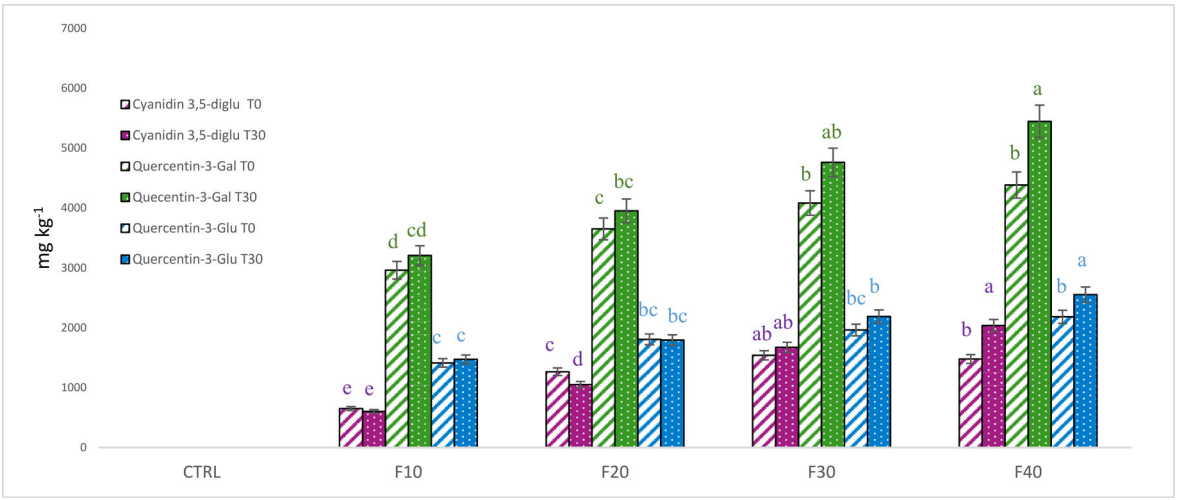

Fig. 1. Concentration (mg kg^{-1}) of the anthocyanins identified in developed fermented milks.

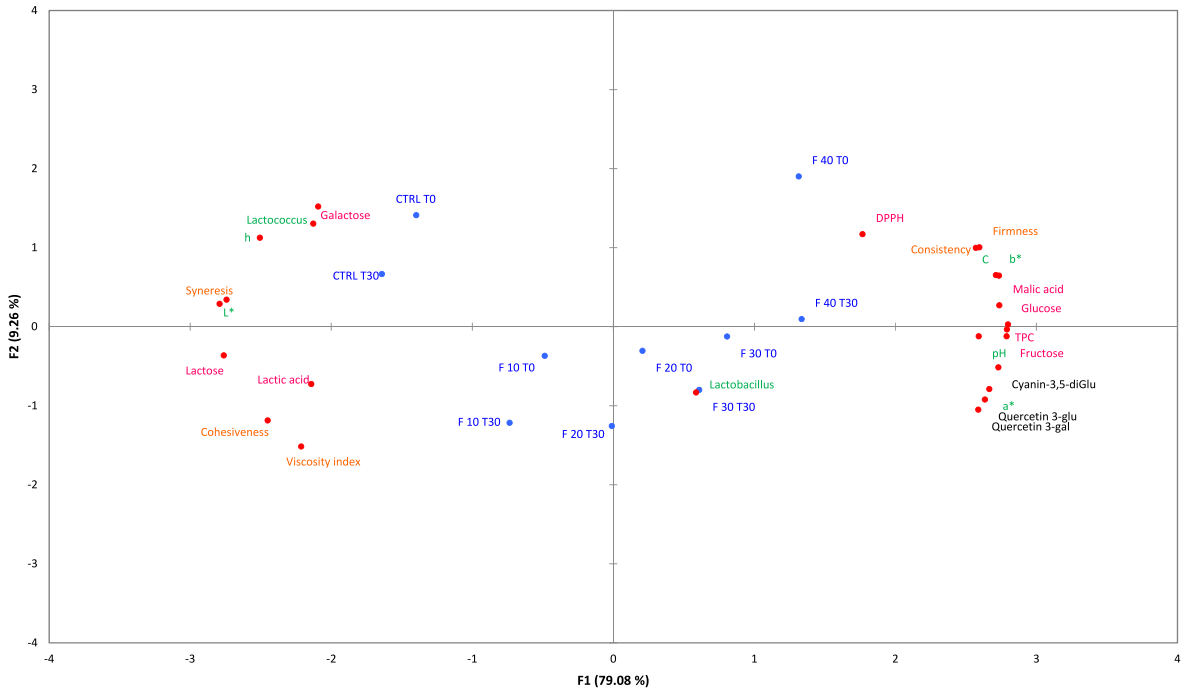

Fig. 2. Principal Component Analysis (PCA) of developed fermented milks and parameters with statistical differences (89 %).

3.5. Flavonoids content

Three flavonoids were identified (2 flavonols and 1 anthocyanin) in the fermented milks (Fig. 1). At T0, quercentin-3-galactoside (mean value of 3770 mg kg^{-1}) was the highest flavonol identified and quantified followed by quercentin-3- glucoside (mean value of 1844 mg kg^{-1}) and cyanidin 3,5-diglucoside (mean value of 1235 mg kg^{-1}). Significant differences were found among samples with different fig percentage at both T0 and T30 (Fig. 1). As expected, any of them was detected in CTRL and as the fig puree content increased, the detected compounds increased. The fig puree incorporated was previously pasteurized, which likely accounts for the absence of the rich anthocyanin profile typically found in fresh figs. These authors Wojdyło et al. (2016) researched phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits and detected the content of cyanidin 3, 5-diglucoside (mean value of 1.0 mg 100 g^{-1} dm) and quercentin-3-galactoside (mean value of 8.3 mg 100 g^{-1} dm) in different cultivars figs. While our results of the pasteurized puree showed contents of cyanidin 3,5-diglucoside (53.9 μg g^{-1}), quercetin 3-galactoside (160 μg g^{-1}) and quercetin 3-glucoside (48.8 μg g^{-1}). In addition, previous research (Sakhale et al., 2015) reported significant losses in anthocyanin content during heating in the fig jam-making process.

Significant differences were found between sampling time in each formulation. It is essential to highlight that a higher content was extracted using the same methodology at T30 compared to samples at T0. The highest amounts of all detected compounds, in particular quercetin-3-galactoside, were found in milks fermented with 40 g 100 g^{-1} fig puree at T30. After 30 days of storage, cyanidin 3,5-diglucoside of FP fermented milks samples kept constant for F10 and F30 formulations, although F20 showed a result decreasing and formulation F40 obtained increased values. For quercetin 3-galactoside, the results increase in all formulations after 30 days of storage. Quercetin 3-glucoside remained constant with formulations F10 and F20 during 30 days of storage while the F30 and F40 formulations showed higher values at T30. The dairy matrix could contribute for stability of some phenolic compounds (Oliveira et al., 2018). Contrary (Du et al., 2022), that studied antioxidant activity in yogurt supplemented with mulberry pomace, reported that especially anthocyanins gradually degraded due to oxidation and utilization during the refrigerated storage. Interactions between proteins and flavonoids like anthocyanins and flavonols have been extensively studied. Higher numbers of hydroxyl groups (-OH) in flavonoids result in stronger hydrogen bonds with proteins, as evidenced other authors (Arts et al., 2002; Li et al., 2023; Yuksel et al., 2010). Caseins, being hydrophobic molecules with regions of negative and positive charge, form stable bonds with flavonoids. Also, due to this factor, the presence of multiple -OH groups and a positive charge, the time stability of cyanidin 3,5-diglucoside is ensured (Trigueros et al., 2014) copigmentation between cyanidin 3,5-diglucoside and studied flavonols through ionic interactions and hydrogen bonds, particularly with quercetin 3-glucoside, results in more stable complexes over time (Cavalcanti et al., 2011). These interactions, observed by Cao et al. (2023) in Dashi blackberry juices, contribute to maintaining anthocyanin concentrations, especially when flavonols like kaempferol, rutin, quercetin, and isoquercetin are involved, mediated by hydrogen bonds and van der Waals forces. In other hand, malic acid was protective in bread samples with grape seed proanthocyanidins, reducing thermal degradation and enhancing final product content (Zhang et al., 2023). In addition, fermenting cultures, notably LAB, influence phenolic compound stability via pH changes and metabolic processes. The specific LAB combination in fig fermented milks production-maintained anthocyanin levels, as seen in blueberry and pomegranate yogurts (Cano-Lamadrid et al., 2017; Ścibisz et al., 2012). Finally, Temperature and sugar content also affect flavonoid stability, refrigeration and sucrose enhance stability (Nikkhah et al., 2007). The increase in bioactive compounds in fermented milks with fig puree shows that lactic acid bacteria have not metabolized the bioactive compounds. These positive results indicate the feasibility of adding fig puree to fermented milks, although there is a need to improve the preservation of bioactive compounds in the fig puree.

3.6. Principal Component Analysis (PCA)

Principal component analysis (PCA) is an important tool used for visualizing the relationship between the analyzed data and its composition. Therefore, the first two principal components (Fig. 2) explained 88.34 % (F1 = 79.08 % and F2 = 9.29 %, respectively) of the total

Table 4
Pearson's correlation coefficient showing the strength of relationship between variables. Values in bold are significant at 95 % confidence limit.

	Malicacid	Lacticacid	Lactose	Galactose	Glucose	Fructose	Cyanin	Quercetin3 – gal	Quercetin3 – glu	TPC	DPPH	L*	a*	b*	C	h	Syneresis	Firmness	Consistency	Cohesiveness	Viscosity	pH	*Lactobacillus*	*Lactococcus*
Malic acid	**1**																							
Lactic acid	**−0.669**	**1**																						
Lactose	**−0.949**	**0.762**	**1**																					
Galactose	**−0.671**	0.422	0.619	**1**																				
Glucose	**0.988**	**−0.711**	**−0.972**	**−0.713**	**1**																			
Fructose	**0.969**	**−0.718**	**−0.949**	**−0.786**	**0.983**	**1**																		
Cyanin	**0.912**	**−0.681**	**−0.946**	**−0.767**	**0.962**	**0.946**	**1**																	
Quercetin 3-gal	**0.864**	−0.553	**−0.849**	**−0.911**	**0.905**	**0.926**	**0.948**	**1**																
Quercetin 3-glu	**0.879**	−0.592	**−0.868**	**−0.911**	**0.918**	**0.942**	**0.953**	**0.998**	**1**															
TPC	**0.968**	**−0.698**	**−0.967**	**−0.75**	**0.98**	**0.987**	**0.948**	**0.926**	**0.939**	**1**														
DPPH	0.593	**−0.865**	−0.602	−0.41	0.58	0.618	0.474	0.411	0.467	0.575	**1**													
L*	**−0.968**	**0.698**	**0.951**	**0.781**	**−0.991**	**−0.981**	**−0.978**	**−0.945**	**−0.955**	**−0.978**	−0.554	**1**												
a*	**0.9**	**−0.636**	**−0.869**	**−0.914**	**0.93**	**0.959**	**0.941**	**0.986**	**0.993**	**0.946**	0.54	**−0.963**	**1**											
b*	**0.932**	**−0.858**	**−0.971**	−0.604	**0.943**	**0.942**	**0.883**	**0.793**	**0.824**	**0.944**	**0.766**	**−0.916**	**0.848**	**1**										
C	**0.964**	**−0.796**	**−0.986**	−0.567	**0.97**	**0.946**	**0.906**	**0.797**	**0.822**	**0.952**	**0.675**	**−0.937**	**0.84**	**0.984**	**1**									
h	**−0.83**	0.582	**0.792**	**0.953**	**−0.867**	**−0.91**	**−0.899**	**−0.982**	**−0.985**	**−0.894**	−0.488	**0.917**	**−0.989**	**−0.771**	**−0.753**	**1**								
Syneresis	**−0.911**	**0.78**	**0.923**	**0.798**	**−0.948**	**−0.973**	**−0.95**	**−0.925**	**−0.942**	**−0.96**	**−0.638**	**0.963**	**−0.957**	**−0.924**	**−0.912**	**0.923**	**1**							
Firmness	**0.934**	**−0.739**	**−0.962**	−0.467	**0.923**	**0.891**	**0.833**	**0.715**	**0.74**	**0.915**	**0.639**	**−0.877**	**0.758**	**0.955**	**0.976**	**−0.658**	**−0.826**	**1**						
Consistency	**0.92**	**−0.732**	**−0.955**	−0.478	**0.909**	**0.881**	**0.82**	**0.716**	**0.741**	**0.911**	**0.639**	**−0.866**	**0.757**	**0.949**	**0.962**	**−0.661**	**−0.812**	**0.996**	**1**					
Cohesiveness	**−0.903**	**0.653**	**0.914**	0.383	**−0.876**	**−0.842**	**−0.772**	**−0.645**	**−0.673**	**−0.875**	−0.585	**0.823**	**−0.692**	**−0.904**	**−0.939**	0.584	**0.785**	**−0.966**	**−0.95**	**1**				
Viscosity	**−0.835**	0.574	**0.861**	0.258	**−0.798**	**−0.75**	**−0.68**	−0.533	−0.562	**−0.793**	−0.534	**0.729**	−0.575	**−0.845**	**−0.886**	0.454	**0.665**	**−0.948**	**−0.939**	**0.979**	**1**			
pH	**0.903**	**−0.681**	**−0.908**	−0.589	**0.939**	**0.88**	**0.944**	**0.823**	**0.829**	**0.875**	0.454	**−0.939**	**0.832**	**0.843**	**0.902**	**−0.766**	**−0.879**	**0.838**	**0.806**	**−0.803**	**−0.718**	**1**		
Lactobacillus	0.104	−0.26	−0.212	−0.108	0.196	0.197	0.282	0.211	0.185	0.199	−0.16	−0.224	0.176	0.133	0.161	−0.178	−0.254	0.159	0.149	−0.082	−0.022	0.318	**1**	
Lactococcus	**−0.657**	0.49	**0.729**	**0.656**	**−0.751**	**−0.72**	**−0.888**	**−0.833**	**−0.818**	**−0.72**	−0.163	**0.793**	**−0.775**	−0.617	**−0.657**	**0.768**	**0.785**	−0.531	−0.506	0.477	0.364	**−0.837**	−0.382	**1**

variation of the experimental data. It can be noted different groups on the figure being a clear one CTRL samples at both T0 and T30 which was characterized by the highest value of L*, content of lactose, galactose, lactic acid, *Lactococcus*, h value and syneresis. Pearson's coefficient between L* coordinate showed negative correlations with glucose and fructose ($r^2 = -0.99, -0.98$, respectively; $p < 0.05$), meaning that the samples with higher percentage of fig puree was less light. Fermented milks without fig puree or low percentage were characterized by low C value which presented positive correlations with lactose and b* ($r^2 = 0.99, 0.98$; $p < 0.05$) (Table 4). As expected, the higher pasteurized fig puree, the higher values of several parameters such as antioxidant capacity, texture parameters DPPH, CIELab coordinates (C and b*), identified and quantified flavonoids, malic acid, glucose, fructose and identified flavonoids. The a* coordinate showed positive correlations with quercetin 3-galactoside ($r^2 = 0.99$; $p < 0.05$), which meant that fermented milks with more content of fig puree had more content of this flavonols. Consistency showed positive correlations with firmness ($r^2 = 1.00$; $p < 0.05$) and viscosity showed positive correlations with cohesiveness ($r^2 = 0.98$; $p < 0.05$).

4. Conclusion

The addition of pasteurized fig puree to the fermented milk showed significant effects on several parameters, such as pH, microbial count, colour, texture, syneresis, sugar content, antioxidant properties and the presence of bioactive compounds. Despite slight variations observed in some parameters during the 30 days of cold storage, overall stability was maintained, indicating the potential of these dairy products. The viability of the lactic acid bacteria was ensured during the shelf life established for the product. In particular, the addition of 30 g 100 g^{-1} to 40 g 100 g^{-1} fig puree improved the texture of the yoghurt, reduced syneresis and increased the presence of bioactive compounds, in particular quercetin-3-galactoside. Fig puree played an important role in improving yoghurt quality and could be used as a valuable ingredient in yoghurt formulations, promoting the value of by-products from *Ficus carica* fruit processing and increasing consumer benefits.

CRediT authorship contribution statement

C. Teruel-Andreu: Writing – review & editing, Writing – original draft, Formal analysis. **N. Jiménez-Redondo:** Formal analysis. **R. Muelas:** Formal analysis. **A. Almansa:** Formal analysis. **F. Hernández:** Writing – review & editing, Methodology, Conceptualization. **M. Cano-Lamadrid:** Writing – review & editing, Supervision, Methodology, Conceptualization. **E. Sendra:** Writing – review & editing, Methodology, Conceptualization.

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Declaration of competing interest

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

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Data availability

Data will be made available on request.

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