



Evaluation of cinnamon (*Cinnamomum cassia* and *Cinnamomum verum*) enriched yoghurt during refrigerated storage

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ABSTRACT

The development of new yoghurt formulations with spices may widen the existing market offer of products matching consumer demands on novel, and "no added sugars" products. The aim of this research was to determine the effect of the addition of *Cinnamomum cassia* (Cs) and *Cinnamomum verum* (Cy) powders to sugar-free yoghurt at 0.5% and 1.5% concentrations. Texture, microbial counts, syneresis, color, antioxidant capacity, total phenols and sensory properties (descriptive and affective analysis) were measured at the beginning (T0) and after 30 days of refrigerated storage (T30). The amount of added cinnamon powder affected yoghurt texture and spontaneous syneresis, concluding that formulations with 1.5% cinnamon were not suitable. The addition of 0.5% cinnamon before yoghurt fermentation allowed the proper fermentation by lactic acid bacteria and preserved yoghurt quality parameters, whereas obtaining high scores on consumer acceptability. Viability of lactobacilli decreased at T30, especially for 1.5% enriched yoghurts.

1. Introduction

Fermented milks are made by the addition of suitable lactic acid bacteria to heat-treated milk, followed by incubation (Savaiano & Hutkins, 2020). Yoghurt is the most popular fermented milk, and it is generally defined as a cultured milk product made using *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp *bulgaricus* (FAO, 2011). Yoghurts are very versatile and can be consumed flavored, sweetened or plain. In order to reach consumer demands on novel, "clean label" and "no added sugars" products, both food scientists and food companies are continuously innovating (García-Burgos, Moreno-Fernández, Alférez, Díaz-Castro, & López-Aliaga, 2020). The search for unique food ingredients, sweeteners, and flavourings is a current global market trend to widen the offer of products fulfilling those needs.

Recently, the application of herbs and spices (powder, fresh, extracts, essential oil) in dairy products has been reported (El-Sayed & Youssef, 2019) and is gaining attention (Tang et al., 2019). Herbs are usually obtained from leaves of the plant while spices come from different seeds, roots, barks, fruit berries, arils, pods, and flowers of plants. Spices and

herbs have been used as flavor, colour and aroma enhancing agents and for preservation of several foods, including dairy products (El-Sayed & Youssef, 2019).

Focusing on spices, spiced flavored yoghurts have been previously developed and many researchers have concluded that bioactivity and functionality of yoghurt can be enhanced through fortification with cinnamon, clove, and lemongrass (Granato et al., 2018). Paying attention to cinnamon, Behrad, Yusof, and Goh (2009) concluded that adding stem barks of cinnamon 10% aqueous extract at 6% did not modify yoghurt fermentation and allowed the growth of *Lactobacillus* spp during refrigerated storage. Others reported that the addition of *Cinnamomum verum* water extract 0.1 g ml⁻¹ in goat, cow and camel milk had no relevant effect on the acidification through fermentation (Bakr & Salih, 2013). Lactic acid bacteria (LAB) growth and proteolytic activity were even increased in the presence of cinnamon. Moreover, Helal and Tagliazucchi (2018) found that adding cinnamon bark powder (*Cinnamomum cassia*) (1.5% w/v) into yoghurt increased the total phenolic content and radical scavenging activity when compared to plain yoghurt. Authors concluded that cinnamon-fortified yoghurt can be

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regarded as a significant source of nutritional bioaccessible polyphenols (Helal & Tagliazucchi, 2018). On the other hand, the addition of 0.5% v/w oleoresins of cinnamon did not negatively affect lactic acid bacteria counts during storability and the antioxidant activity was constant during shelf-life. The products obtained a sensory acceptance over 70% (Ilupapalayam, Smith, & Gamlath, 2014). Also, Shori and Baba (2011) reported that *Cinnamomum verum* bark at 10% aqueous extract (0.1 g ml^{-1}) enhanced techno-functional (acidification, proteolysis) and functional (total phenolic content, and antioxidant activities) properties (Shori & Baba, 2011).

Among *Cinnamomum* genus, four species are usually called cinnamon: i) *Cinnamomum loureiroi* or Vietnamese cinnamon; ii) *Cinnamomum burmanni* or Indonesian cinnamon; iii) *Cinnamomum zeylanicum* or *Cinnamomum verum*, and iv) *Cinnamomum aromaticum* or *Cinnamomum*

cassia (Nabavi et al., 2015). The last two species are the most widely used in the world. Several neuroprotective properties of cinnamon such as anti-neuro-inflammatory and antioxidant effects have been recently associated to cinnamon (Ayati, Emami, Guillemin, Karamacoska, & Chang, 2021). Also, cinnamon extracts (powders, oils) have shown antimicrobial properties (Brnawi, Hettiarachchy, Horax, Kumar-Phillips, & Ricke, 2019; Jeong, Kim, Han, & Choi, 2021), which has to be taken into account when adding cinnamon to fermented milk as it may impair the growth and survival of lactic acid bacteria. Therefore, when developing cinnamon enriched yogurts proper lactic acid bacteria counts should be assured during yoghurt shelf-life.

Considering all the above-mentioned aspects, the aim of this research was to determine the effect of the addition of *Cinnamomum Cassia* (Cs) and for such purpose, techno-functional (texture, microbial counts,

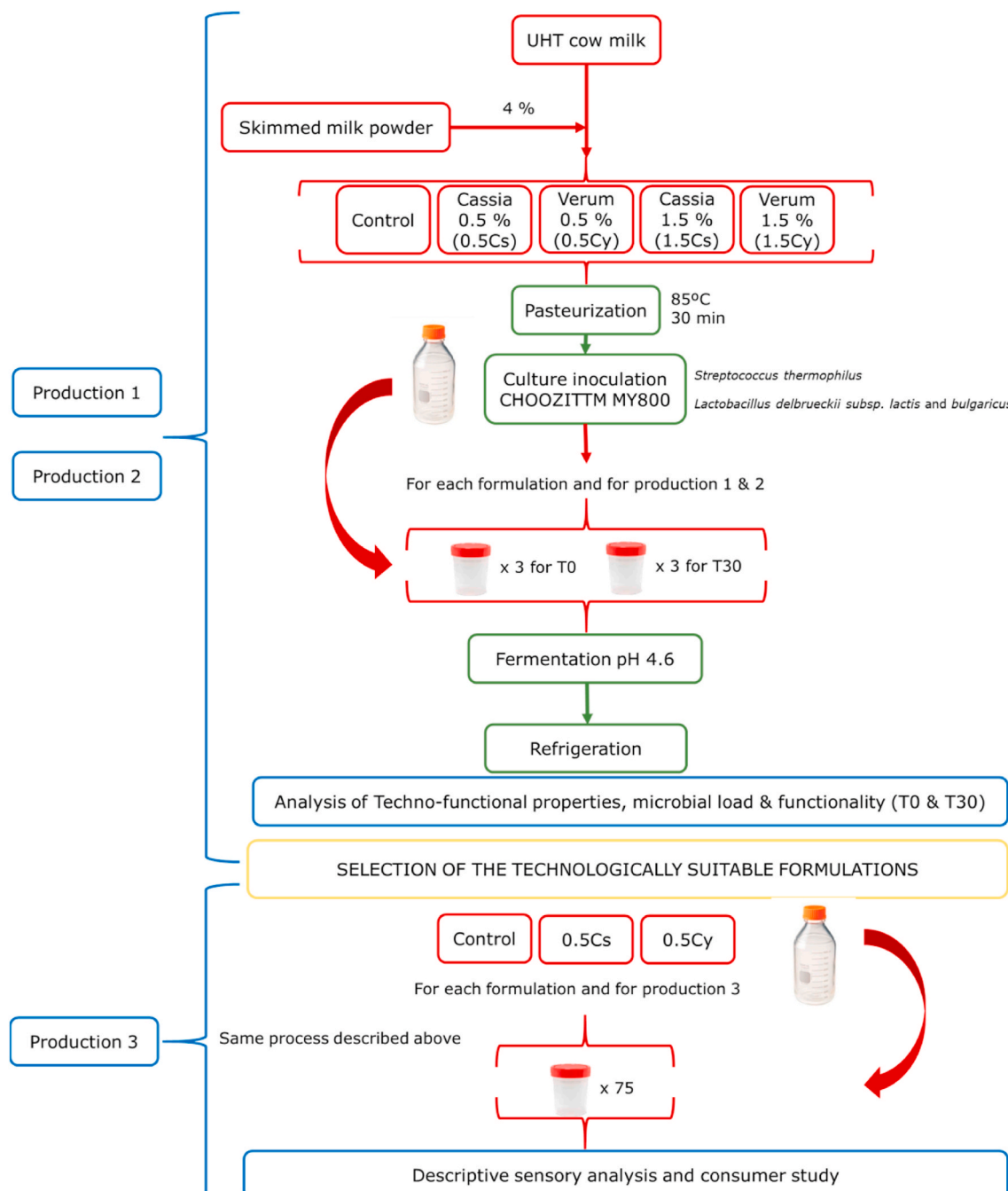


Fig. 1. Experimental design.

syneresis, color), functional (antioxidant capacity and total phenols) and sensory (descriptive and affective analysis) properties were determined at the beginning (T0) and after 30 days of refrigerated storage (T30).

2. Materials and methods

2.1. Cinnamon material, milk and starter

Cinnamomum cassia (Cs) and *Cinnamomum verum* (Cy) barks were purchased at a local supermarket. Both species of cinnamon were grounded in a Moulinex grinder, model AR110830 (Alençon, France) for 40 seconds and sieved (sieve mesh count: 0.16 mm). Commercial Ultra-Heat Treatment (UHT) cow milk was used for yoghurt manufacturing with the addition of 4% skimmed cow milk powder (Iparlat, Renedo de Piélogos, Cantabria, Spain). Freeze-dried lactic starter was used following manufacturer indications (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (CHOOZIT™ MY800 LYO 5 DCU, Rhodia Food-Danisco A/S, Sassenage, Francia).

2.2. Yoghurt manufacture

Yoghurt was produced following the manufacture method developed by Trigueros, Wojdylo, and Sendra (2014). Fig. 1 shows the experimental design and the specifications of each processing step. Formulations tested were: i) Cs0.5 (*Cinnamomum cassia* 0.5%), ii) Cs1.5 (*Cinnamomum cassia* 1.5%), iii) Cy0.5 (*Cinnamomum verum* 0.5%), and, iv) Cy1.5 (*Cinnamomum verum* 1.5%) and Control yoghurt with no cinnamon. Three independent replicates/productions of each formulation were run. The blend of the ingredients was done in 1000 mL Pyrex™ flasks followed by VAT pasteurization (85 °C, 30 min). The fermentation occurred in sterile sample containers (PP) (60 mL) at 43 °C in an incubator (Selecta, Barcelona, Spain) (pH and acidity were monitored till pH 4.6 was reached), afterwards yoghurts were refrigerated at 4 °C. Samples were taken at 24 h (T0) and 30 days (T30) of refrigerated storage. Three individual replicates were analyzed for each batch and yoghurt type. Once technological properties were evaluated, the most suitable formulations were manufactured for sensory analysis (production 3): control, Cs0.5 and Cy0.5. Seventy-five containers of each selected formulation were used for descriptive and hedonic analysis.

2.3. Techno-functional properties analysis

Penetration test was performed with a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, England) with a 5 kg load cell. Constant speed penetration tests were performed directly on cylindrical containers (4.5 cm diameter, 4 cm height) (Trigueros, Pérez-Álvarez, Viuda-Martos, & Sendra, 2011). All instrumental texture analyses were conducted at 8 °C and spontaneous syneresis was removed previously. This is a “destructive” test as no structure recovery is allowed. A cylindrical probe 10 mm diameter ebonite (P-10) was introduced 15 mm into the samples at a speed of 1 mm s⁻¹. Triplicate measures for each yoghurt were performed. Gel stability was visually assessed after incubation (Spontaneous syneresis), and determined by quantifying the volume of whey removed from curd after centrifugation (syneresis) (Morgan et al., 2003).

The CIEL*a*b* color space of yoghurt was studied, and the following color coordinates were evaluated: lightness (L*), redness (a*, green-red coordinate), and yellowness (b*, blue-yellow coordinate). Color determinations were made at 12 ± 2 °C by means of a Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer, with a liquid accessory CR-A70 (Minolta Camera Co., Osaka, Japan), with illuminant D65 and an observer of 10°. The equipment was daily calibrated with the white plate provided by Minolta. Additionally, pH was also determined at 12 °C (pH Basic 20 instrument, Crison, Barcelona, Spain) and calibrated with pH standards (4 and 7). The probe was inserted

following the instructions of the equipment (5 cm). MRS agar was used for Lactobacilli counts (incubated at 37 °C, microaerophila, 48 h), M17 for Lactococci (incubated at 30 °C, aerobiosis, 24 h). Three replicates were run for pH and microbial counts, and nine for color. Organic acids and sugars of fermented milk samples were analyzed by High-Performance Liquid Chromatography (HPLC) (Trigueros, Sayas-Barberá, Pérez-Álvarez, & Sendra, 2012).

2.4. Functional properties

Total phenolics were extracted according to the protocol by Li et al. (2009) and quantified in triplicate using the Folin–Ciocalteu reagent, and results were expressed as mg gallic acid equivalent per g of fresh weight (fw). The antioxidant capacity (DPPH•) was carried out following Brand-Williams, Cuvelier, and Berset (1995) and results were expressed as mmol Trolox per g of fresh weight (fw).

2.5. Sensory analysis

A trained panel consisting of 10 highly trained panelists from the research group Food Quality and Safety Group (Universidad Miguel Hernández de Elche, UMH Orihuela, Alicante, Spain) conducted the descriptive sensory analysis in one session. The panel was selected and trained following the ISO standard 8586-1. The scale ranged from 0 (no intensity) to 10 (extremely high intensity) with 0.5 increments. Each panelist tested ~25 mL of each sample, coded with 3-digit randomized numbers. Water and unsalted crackers were provided to panelists to clean the palate between samples.

A sample group of 60 consumers was recruited at UMH (Spain) aged between 18 and 70 years. The main requirement for their recruitment was that they consumed regularly yoghurt and cinnamon. The consumer study was conducted at the UMH facilities in Orihuela. Consumers were asked about their global satisfaction degree using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely), together with questions regarding attributes intensity using a Just About Right (JAR) scale. Each consumer was served ~25 mL of each sample coded with 3-digit numbers, together with the questionnaire. Water and unsalted crackers were provided to consumers between samples for palate cleaning. Consumers were asked to evaluate appearance of the intact sample and then gently stir the sample with a spoon, ten times clockwise and ten times anticlockwise, before further evaluation.

2.6. Statistical analyses

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 using first “formulation” and second “storage” as factors. Tukey test was used for means comparison (95% confidence level). Principal component analysis (PCA regression map) was conducted to project the samples depending on the techno-functional parameters and microbial load. Penalty analysis using XLSTAT Premium 2016 (Addingsoft, Barcelona, Spain) was conducted to provide extra information about the possible improvements of some samples.

3. Results and discussion

3.1. Texture parameters and gel stability

Table 1 shows texture parameters of developed yoghurts. Among formulations, significant differences ($p < 0.001$) were observed in “Distance for Maximum Force” (Distance for Fmax), “maximum force” (Fmax), and “Total Work for penetration”. Cs1.5T0 and Cy1.5T0 yoghurts had significantly ($p < 0.001$) lower “Distance for Fmax” and “Total work” values than control, 0.5CsT0 and 0.5CyT0. Fmax for

Table 1

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

Sample	Distance for Fmax (mm)	Fmax (g)	Total work (g × mm)	Syneresis (%)	Spontaneous syneresis (%)
ANOVA and Tukey test among formulation ^{∞#}					
	***	***	***	***	***
ControlT0	14.82 ± 0.17 ^a	25.01 ± 0.94 ^c	280.35 ± 16.79 ^b	49.12 ± 1.01 ^a	nd [±]
0.5CsT0	14.91 ± 0.057 ^a	28.72 ± 1.3 ^b	346.23 ± 15.65 ^a	38.21 ± 2.17 ^b	nd
0.5CyT0	14.85 ± 0.11 ^a	22.46 ± 0.89 ^d	270.35 ± 7.97 ^b	50.76 ± 0.86 ^a	nd
1.5CsT0	3.48 ± 2.23 ^b	50.34 ± 6.03 ^a	97.34 ± 66.18 ^d	31.35 ± 2.53 ^b	23.04 ± 4.31 ^a
1.5CyT0	7.42 ± 5.94 ^b	15.02 ± 12.37 ^c	172.91 ± 94.86 ^c	52.96 ± 1.84 ^a	0.81 ± 0.96 ^b
ANOVA between storage time (T0 cv T30) ^μ					
	NS	***	***	***	**
ControlT30	14.91 ± 0.083	28.94 ± 1.69	323.17 ± 14.1	31.48 ± 2.03	0.33 ± 0.66
	NS	NS	NS	***	
0.5CsT30	14.89 ± 0.13	29.51 ± 0.98	349.28 ± 11.96	26.57 ± 1.71	nd
	NS	NS	NS	***	**
0.5CyT30	14.86 ± 0.15	23.12 ± 2.59	266.84 ± 25.87	36.49 ± 2.38	0.56 ± 0.55
	NS	NS	NS	***	NS
1.5CsT30	2.86 ± 0.54	48.09 ± 3.75	78.98 ± 21.75	19.7 ± 1.66	21.02 ± 3.91
	**	NS	NS	***	**
1.5CyT30	12.44 ± 4.83	28.84 ± 11.34	237.28 ± 102.96	29.8 ± 6.8	5.82 ± 7.09

^α Fmax = maximum force; [∞] NS = not significant ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [#] Among formulation, values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test; ^μ Between T0 and T30, ANOVA test indicated if there were significant differences; ±nd = no detected.

penetration is a measure of firmness. Such parameters provide information on gel structure, a yogurt with poor structure will show low Fmax, low Total Work, and a Distance for Fmax equal to the maximum distance (15 mm). All of them showing a low resistance to penetration. A yogurt with some elastic or more firm behavior will offer some resistance to penetration (maybe Fmax detected before maximum distance and higher Work for penetration). As observed, these parameters were mainly affected by cinnamon concentration, being the concentration 0.5% the most similar to the control samples for these parameters. On the other hand, Cy yoghurts (0.5 and 1.5%) had significantly ($p < 0.001$) lower Fmax values than the respective concentration of Cs yoghurts and even control yoghurt. It is important to highlight that cinnamon provides characteristics of viscous flow or mucilaginous (mucilage is a thick, gluey substance produced by plants) behavior during processing most likely due to the carbohydrates with degree of polymerization over 200 such as cellulose, hemicelluloses, specifically arabinoxylan, β-glucan and pectin (Jean-Pederson & Nowakowski, 2017). It was observed that both types of cinnamon gave different texture characteristics in developed yoghurts. Arabinoxylans form highly viscous aqueous solutions and generally have a strong water-holding capacity. If hemicelluloses such as arabinoxylans are present in cinnamon, this may be one reason why viscosity is an issue during processing. No data of

differences of these compounds between Cs and Cy was found, however we postulate that Cs is expected to have higher contents of such compounds given the higher viscosity of Cs yoghurts as compared to Cy. Another relevant fact is the dramatic difference among texture of 0.5 vs 1.5 cinnamon yoghurts. The high number of particles in 1.5% cinnamon yoghurts may have impaired yoghurt structure through physically interrupting the network and probably also due to protein:phenols interaction, the polyphenols of cinnamon powder react with proteins, destabilizing the casein micelles (spontaneous syneresis), specially when 1.5% (Cs much higher than Cy) was added. Our results agree with previous studies which indicate that Cs presented higher content of total phenols, specially coumarin, than Cy (Silva, Bernardo, Singh, & Mesquita, 2019).

Texture modifications (T0 vs T30) occurred after 30 days of refrigerated storage only in control yoghurt: a significant increase of Fmax and "Total Work". The shrinkage of the gel (due to syneresis) may have reinforced the protein network responsible for such enhanced firmness. Microbial growth and metabolism of yoghurt components during storage has been previously reported to be the key factor with regard to texture changes (Duboc & Mollet, 2001), however no textural differences were observed on cinnamon yoghurts due to storage.

After refrigerated storage, spontaneous syneresis appeared in controlT30 and 0.5CyT30 (less than 1%). No significant difference was found due to storage time when Cs was used (1.5CsT0 and 1.5CsT30), whereas a significant increase in syneresis was observed between 1.5CyT0 and 1.5CyT30. Once spontaneous syneresis was removed, syneresis by centrifugation was determined. Components of Cs enhanced water holding capacity (less % of syneresis than Cy) since they contribute to the mesh effect in the three-dimensional network of the gel formed in yoghurt (higher Fmax). Previous studies indicated that the strength of the protein network increases when lactic acid and exopolysaccharides content increase due to lactic acid bacteria metabolism (Lubbers, Decourcelle, Vallet, & Guichard, 2004). The highest Fmax (after removing spontaneous syneresis) belongs to the formulation (1.5Cs) with the highest content of lactic acid and also concentrated due to the spontaneous syneresis. Casein structure could have been affected by cinnamon components, pasteurization and further set gel formation. The main cinnamon compounds are procyanidin type-A polymers, cinnamic acid, cinnamaldehyde, and coumarin. The main difference of bioactive compounds between Cs and Cy is that Cs presents higher coumarin content (Silva et al., 2019). Therefore, adding different cinnamon species develops different textural properties. Also, the addition of Cy has been reported to increase proteolysis in cow milk yoghurt (Shori & Baba, 2011), an excessive casein hydrolysis may result in a too-soft final product (Amani, Eskandari, & Shekarforoush, 2017).

3.2. Colour coordinates

Table 2 shows the results of the color coordinates. As expected L^* parameter was affected by the incorporation of 0.5 and 1.5% of both species of cinnamon, being lower in formulations with 1.5%. Thus, the brown color of the cinnamon considerably reduced yoghurt lightness as the cinnamon concentration increased (from 97.5 to less than 82.5). Also, L^* was significantly reduced during refrigerated storage in all formulations (more than 10 units), except for 1.5Cs. According to García-Pérez et al. (2005), L^* in fermented milk decreases as the pH decreases due to gelation, in the present study the addition of cinnamon also contributed to the decreased lightness.

With respect to the parameter a^* , it increased with the concentration of added cinnamon (from -008 to 5.2). Also, it seems that Cs presented higher values than Cy formulations. b^* coordinate had the same behavior. This phenomenon occurs due to the color characteristics of each specie, for instance *C. verum* (Cy) is characterized by a light-yellowish brown colour, while *C. cassia* (Cs), has a darker brown colour (R1151/2012, 2020/C208/08, 2020). During refrigerated storage, a^* coordinate significantly decreased (Table 1). It can be

Table 2

CIE_{Lab} color parameters (L^* , a^* and b^*), total phenols content (TPC) and anti-oxidant capacity (DPPH assay) of developed yoghurts at T0 (24 h) and T30 (30 days, refrigerated storage).

Sample	L^*	a^*	b^*	TPC ^a (mg eq gallic acid g ⁻¹)	DPPH [*] (mmol Trolox g ⁻¹)
ANOVA and Tukey test among formulation^{∞#}					
	***	***	***		NS
ControlT0	97.50 ± 1.07 ^a	−0.08 ± 0.04 ^e	2.43 ± 0.12 ^e	nd ±	0.17 ± 0.02
0.5CsT0	88.13 ± 0.35 ^b	4.04 ± 0.28 ^c	8.60 ± 0.32 ^c	nd	0.17 ± 0.02
0.5CyT0	88.99 ± 0.5 ^b	3.37 ± 0.14 ^d	5.96 ± 0.24 ^d	nd	0.19 ± 0.01
1.5CsT0	81.38 ± 0.52 ^c	5.18 ± 0.23 ^a	15.06 ± 0.31 ^a	0.36 ± 0.11 ^a	0.19 ± 0.08
1.5CyT0	82.16 ± 0.6 ^c	4.53 ± 0.012 ^b	11.06 ± 0.15 ^b	0.62 ± 0.27 ^a	0.18 ± 0.09
ANOVA between storage time (T0 cv T30)^μ					
	***	***	***		NS
ControlT30	83.73 ± 0.67	−2.71 ± 0.034	7.35 ± 0.082	nd	0.14 ± 0.02
0.5CsT30	75.44 ± 0.76	1.19 ± 0.33	12.13 ± 0.17	nd	0.15 ± 0.07
0.5CyT30	76.74 ± 0.27	0.55 ± 0.24	9.92 ± 0.42	nd	0.15 ± 0.05
	NS	**	***	NS	NS
1.5CsT30	82.72 ± 17.19	1.70 ± 2.06	9.32 ± 7.49	0.39 ± 0.13	0.15 ± 0.04
	***	***	***	NS	NS
1.5CyT30	69.32 ± 1.76	2.35 ± 0.25	14.12 ± 0.73	0.57 ± 0.24	0.15 ± 0.06

^a Fmax = maximum force; [∞] NS = not significant ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [#] Among formulation, values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test; ^μ Between T0 and T30, ANOVA test indicated if there were significant differences; ±nd = no detected.

appreciated that b^* changed during storage time for all fermented milks, but no clear tendency was observed, both changes in color coordinates maybe due to the desestabilization of the gel structure with the release of milk whey characterized by a greenish color.

3.3. Microbial counts and metabolic products

Table 3 shows the pH, microbial load together with metabolic products after fermentation (lactose, galactose, and lactic acid). All formulations reached pH < 4.6 (Table 3). However, control formulation (ControlT30) showed a significant pH reduction after refrigeration, related to the higher content of lactic acid and galactose and a reduction of lactose. Kailasapathy, Harmstorf, and Phillips (2008) indicated that pH reduction during storage together with an increase of organic acids affected negatively cell viability of lactic acid bacteria. It might be specified that fermentation process (reaching pH 4.6) took 8 h in Control and formulations with 0.5% cinnamon as previous studies indicate (Cândido de Souza, Souza do Amaral, & Lima da Silva Bernardino, 2021). Trigueros et al. (2014) indicated that the time for reaching pH 4.6 was 4 h. When 1.5% of cinnamon (Cs and Cy) was added, fermentation process took 22 h, being no technologically suitable and showing a clear inhibition of lactic acid bacteria. The main reason could be due to the antibacterial effects (mainly bacteriostatic ones) of cinnamon constituents such as cinnamaldehyde and eugenol, being higher 1.5% formulations (Nabavi et al., 2015). Behrad et al. (2009) concluded that the addition of 10% of 6% aqueous cinnamon stem barks extract did not affect the yoghurt fermentation and the viability of *Lactobacillus* spp during refrigerated storage. Also, Shori and Baba (2011) reported that *Cinnamomum verum* bark at 10% of the aqueous extract (0.1 g mL⁻¹)

Table 3

pH, microbial load (*Lactobacillus* and *Lactococcus*) and metabolic products (Lactic acid, galactose and lactose) of developed yoghurts at T0 (24 h) and T30 (30 days, refrigerated storage).

Sample	pH	Lab [‡]	Lac [≈]	Lactic acid	Galactose	Lactose
		Log CFU g ⁻¹			g/100 g	
ANOVA and Tukey test among formulation^{∞#}						
	NS	**	***	**	*	NS
ControlT0	4.50 ± 0.01 [‡]	7.11 ± 0.06 ^{ab}	8.74 ± 0.05 ^{ab}	0.71 ± 0.15 ^b	0.60 ± 0.08 ^b	2.85 ± 0.67
0.5CsT0	4.50 ± 0.02 [‡]	7.17 ± 0.35 ^{ab}	9.01 ± 0.16 ^a	0.72 ± 0.08 ^b	0.64 ± 0.04 ^b	3.25 ± 0.27
0.5CyT0	4.50 ± 0.02 [‡]	6.82 ± 0.20 ^b	9.01 ± 0.26 ^a	0.89 ± 0.03 ^{ab}	0.70 ± 0.05 ^{ab}	3.24 ± 0.32
1.5CsT0	4.10 ± 0.06 [∞]	7.54 ± 0.07 ^a	8.26 ± 0.14 ^b	0.97 ± 0.04 ^a	0.73 ± 0.07 ^a	2.69 ± 0.08
1.5CyT0	4.12 ± 0.09 [∞]	7.32 ± 0.19 ^a	8.91 ± 0.22 ^{ab}	0.73 ± 0.08 ^b	0.55 ± 0.08 ^c	2.53 ± 2.91
ANOVA between storage time (T0 cv T30)^μ						
	*	*	NS	*	*	*
ControlT30	4.02 ± 0.06	6.23 ± 0.67	8.87 ± 0.07	0.98 ± 0.05	0.86 ± 0.11	2.76 ± 0.12
0.5CsT30	4.44 ± 0.04	6.27 ± 0.15	8.88 ± 0.06	0.77 ± 0.03	0.70 ± 0.02	2.99 ± 0.07
0.5CyT30	4.10 ± 0.17	6.11 ± 0.17	8.77 ± 0.10	0.84 ± 0.02	0.74 ± 0.02	2.94 ± 0.06
1.5CsT30	4.22 ± 0.09	6.28 ± 0.46	8.34 ± 0.28	0.92 ± 0.1	0.80 ± 0.10	3.32 ± 0.58
1.5CyT30	4.24 ± 0.13	6.19 ± 0.24	5.73 ± 0.83	0.70 ± 0.05	0.68 ± 0.05	2.87 ± 0.15

[‡] Lab: *Lactobacillus*; ≈ Lac: *Lactococcus*; [∞] NS = not significant ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [#] Among formulation, values followed by the different letter within the same column were significant different ($p > 0.05$), Tukey's multiple-range test; ^μ Between T0 and T30, ANOVA test indicated if there were significant differences; [‡] Reached time: 8 h; [∞] Reached time: 22 h.

enhanced techno-functional (acidification, proteolysis) properties. Contrary, Bakr et al. (2013) found no differences on the acidification when 0.1 g mL⁻¹ *Cinnamomum verum* aqueous extract was added in goat, cow and camel milk (Bakr & Salihin, 2013). Counts detected on MRS mainly are attributable to the inoculated LAB culture, while counts detected on M17 to the inoculated Streptococci (LAC) form the culture. Microbial counts were >6.5 Log LAB UFC g⁻¹ and >8.0 Log LAC UFC g⁻¹. The effect of cinnamon on LAB counts was previously reported, resulting in lower LAB counts in cinnamon-yoghurts (9.5) on day 0 of storage compared to plain-yoghurt (13.0) (Behrad et al., 2009). Contrary, Bakr and Salihin (2013) observed that cinnamon extracts stimulated LAB counts. In the present study, microbial counts were affected by percentage of cinnamon powder, not by cinnamon species. Microbial counts were also affected by refrigerated storage: at day 1 the highest counts of LAB were obtained, and then, significantly decreased at day 30 in all formulations. Previous studies indicated that LAB counts were reduced from day 7 to day 28 of storage for plain-yoghurt and

cinnamon-yoghurt with the fastest rate occurred in plain-yoghurt (Behrad et al., 2009). Other study reported no negative effects on microbial counts during refrigerated storage when 0.5% v/w oleoresins of cinnamon were added (Illupapalayam et al., 2014). On the other hand, no significant changes of LAC counts were observed, except for 1.5Cy formulation which LAC counts were reduced <6 .

3.4. Functionality

The reason of the TPC difference between 1.5Cs and 1.5 Cy could be the higher content of TPC in Cy as compared with Cs cinnamon according to the scientific literature (Lopes et al., 2022). Helal and Tagliazucchi (2018) found an increase of the TPC when 1.5% Cs cinnamon bark powder was added, (Helal & Tagliazucchi, 2018). when compared to natural yoghurt. It is important to highlight that the culture used in that study contained only *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. They showed that 34.7% of the total phenolic compounds present in the cinnamon water extract were recovered in the cinnamon-fortified yoghurt indicating that the remaining compounds were actually bound to milk proteins. Also, Shori and Baba (2011) reported that *Cinnamomum verum* bark at 10% of the aqueous extract (0.1 g mL^{-1}) enhanced the functional (total phenolic content, and antioxidant activities) properties (Shori & Baba, 2011).

The DPPH $^{\bullet}$ antioxidant capacity was not affected by the addition of cinnamon ingredient. The presence of DPPH $^{\bullet}$ activity in control formulation is because of the bioactive peptides present in milk and yoghurt (Lamothe, Guérette, Dion, Sabik, & Britten, 2019; Nguyen & Hwang, 2016). It is important to highlight that DPPH $^{\bullet}$ activity decreased after refrigerated storage, probably due to enhanced interactions protein-phenols during storage. Future research should be focused on the effect of storage temperature, cinnamon species and concentration on DPPH values.

3.5. Principal component analysis (PCA)

For an easy visualization of the relationships among all variables of the developed cinnamon yoghurts, a PCA was run for all five samples, including only significantly different variables: textural properties, gel stability parameters, color coordinates, microbial load, lactic acid, galactose, and TPC. Fig. 2 shows the two principal components which explained 81.93% of the samples variation. As observed, yoghurts at 0.5% cinnamon concentration were grouped together with the control

sample and were characterized by harder texture and a lighter color. On the contrary, the yoghurts formulation of 1.5% cinnamon were grouped separately and far from the first group and from each other. As observed *C. cassia* led to yogurts with a higher lactic acid and galactose, but with a higher spontaneous syneresis parameter that affects the technological properties of the developed yoghurt. On The other hand, *C. verum* helped to increase the red-yellow color coordinates and the functionality of the samples.

3.6. Sensory analysis

One of the main attributes driving to consumers acceptance of dairy products is the texture properties, specially firmness and creaminess (Duboc & Mollet, 2001). It is essential to highlight that only suitable techno-functional formulations were used for sensory analysis: control, 0.5Cs and 0.5Cy. It is noteworthy that none of the samples had measurable off-flavours notes, supporting the high quality of the products.

As to descriptive sensory analysis, control sample presented a flat (no complex) sensory profile, with their predominant attribute being dairy, firm and sour (Fig. 3). The profile changed considerably when cinnamon was added; this step enriched the sensory complexity by increasing color, cinnamon ID, spicy ID, sweetness, and creaminess. The incorporation of Cs and Cy into yoghurt revealed that it has more creaminess than control sample due to the presence of soluble polysaccharides, namely highly branched arabinoxylans as above mentioned. The high viscosity conferred to food products by cinnamon could be a technological problem in for other applications in the food industry, but it maybe valuable for yogurts. Previous studies indicated that a treatment to partially hydrolyse arabinoxylans (enzymatic and acid hydrolysis) to decrease its viscosity could be a good choice. Firmness was reduced by adding Cs and Cy at 0.5%.

Regarding consumer evaluation, only liking of appearance before blending, and firmness and viscosity after blending presented significant differences (Table 4). No differences in overall liking were observed. In addition to overall liking and liking of specific attributes, some *Just About Right* (JAR) questions were asked during the consumer study (Fig. 4). For a better understanding of the relationships between JAR scores and consumers linking, penalty analysis was conducted (Cano-Lamadrid et al., 2020; Cano-Lamadrid, Vázquez-Araújo, Sánchez-Rodríguez, Wodyto, & Carbonell-Barrachina, 2018; Lipan et al., 2019). Fig. 4 shows the proportion of consumers' opinion plots against

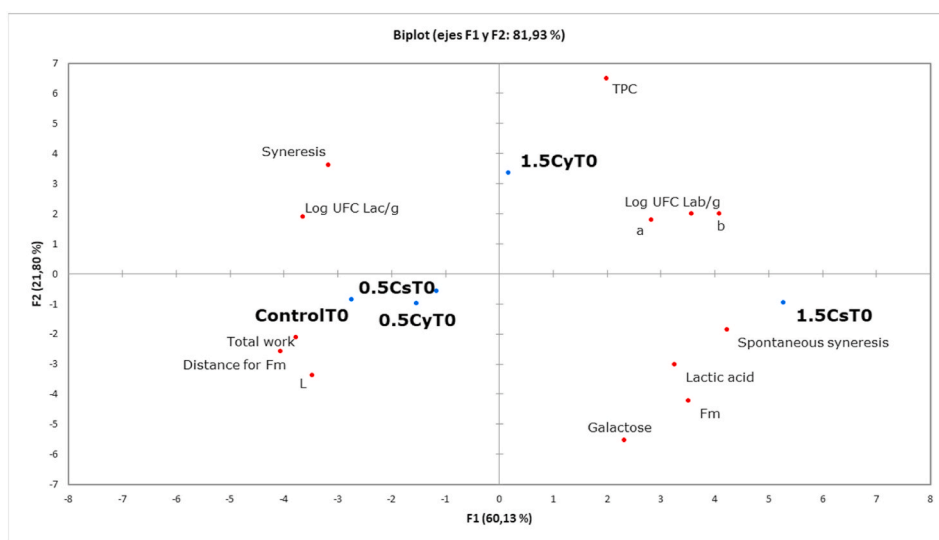


Fig. 2. PCA scores plot showing the relationship among techno-functional parameters and microbial load of developed yoghurts at 0 days (24 h) of cold storage time at 4 °C.

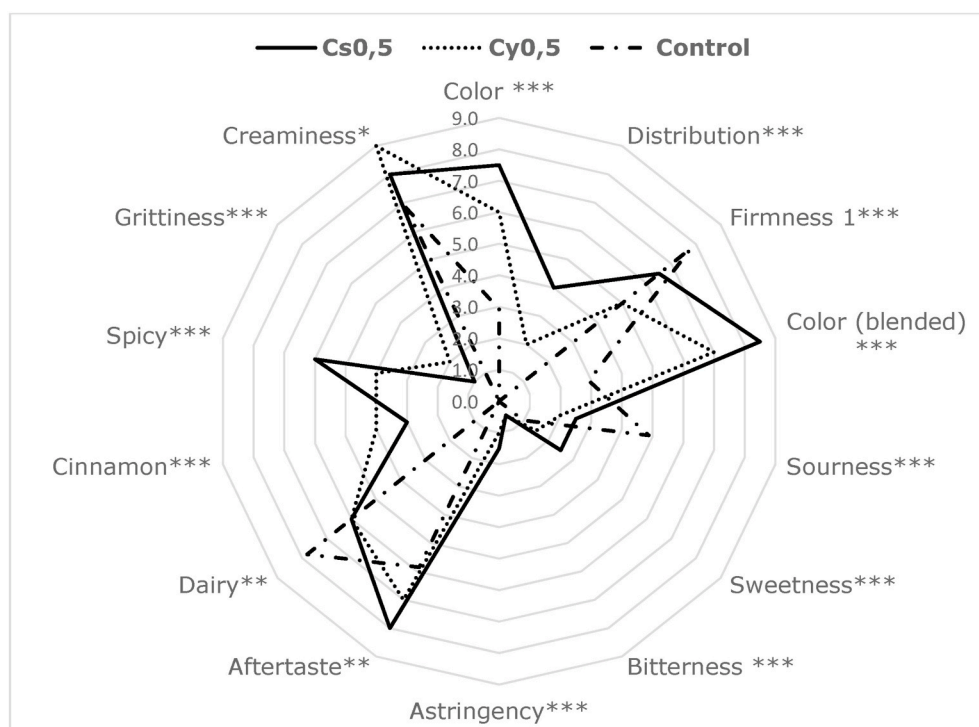


Fig. 3. Descriptive Sensory Analysis of selected developed yoghurts.

Legend: NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

Legend:

NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$,

0.01, and 0.001, respectively.

Table 4

Mean scores and ANOVA of developed yoghurts at T0 (24 h) and T30 (30 days, refrigerated storage) for appearance, flavor notes, basic taste, and overall liking for consumers.

	ANOVA ^{∞#}	Cs0.5	Cy0.5
Appearance	**	5.8 ± 0.5 ^b	7.0 ± 0.5 ^a
Cinnamon at the bottom	NS	6.2 ± 0.5	6.4 ± 0.5
Firmness	**	7.0 ± 0.5 ^a	5.8 ± 0.5 ^b
Creaminess	NS	6.9 ± 0.5	6.4 ± 0.5
Appearance after blending	NS	6.4 ± 0.5	6.3 ± 0.5
Colour	NS	6.6 ± 0.5	6.7 ± 0.5
Viscosity	**	6.2 ± 0.5 ^a	5.1 ± 0.5 ^b
Particles	NS	6.0 ± 0.5	5.8 ± 0.5
Sourness	NS	5.5 ± 0.5	5.4 ± 0.5
Sweetness	NS	5.4 ± 0.5	5.2 ± 0.5
Cinnamon	NS	6.2 ± 0.5	6.1 ± 0.5
Aftertaste	NS	6.0 ± 0.5	5.4 ± 0.5
Overall liking	NS	5.9 ± 0.5	5.8 ± 0.5

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

the mean Penalty. The attributes susceptible of improvement were those that had the greatest negative impact on the sample liking for at least 20% of consumers and caused a drop of at least 1 point for liking. Results of the penalty analysis indicated the clear need of improvement of both formulations. 0.5Cs needed improvement of its sourness, presence of particles and aftertaste because of the excessive intensity. It was also shown that sweetness needed to be increased in this sample but it is essential to highlight that although 77% of consumers eat natural yoghurt (no added-sugar), 29% of the consumers do not sweeten it, while 31%, 13%, 25% and 12% of consumers sweeten natural yoghurt

with sugar, sweeteners, honey and/or cinnamon, respectively. According to consumers, no improvement of cinnamon attribute was necessary in 0.5Cs. On the other hand, 0.5Cs was penalized by presenting low intensities of sourness, viscosity, firmness, creaminess, and aftertaste.

4. Conclusions

The amount and species of cinnamon powder added affects the technological properties of yoghurts (the internal structure of the gel and spontaneous syneresis), concluding that formulations with 1.5% cinnamon (Cs and Cy) are not suitable. Among both species of cinnamon, there are differences in color, microbial load, percentage of syneresis by centrifugation and sensory attributes. The addition of 0.5% of cinnamon (Cs and Cy) before yoghurt fermentation does not affect the fermentation process of the lactic acid bacteria nor quality parameters. These formulations are suitable for obtaining no-added sugar cinnamon yoghurts with lactic bacteria that meet viability requirements during 30 days of refrigeration storage.

Set yoghurts have been manufactured in the present study, from a pasteurized cinnamon-milk blend. Further studies should be conducted evaluating cinnamon addition after milk pasteurization, even after fermentation to evaluate the possibility of enhanced yoghurt functionality (total phenolics and antioxidant activity). The present is the first study reporting the use of different cinnamon species commonly available in the food market and widely consumed. The knowledge provided by the present study may allow the food industry to develop new products meeting the requirements of non. added sugars and iwht enhanced technofuntional properties.

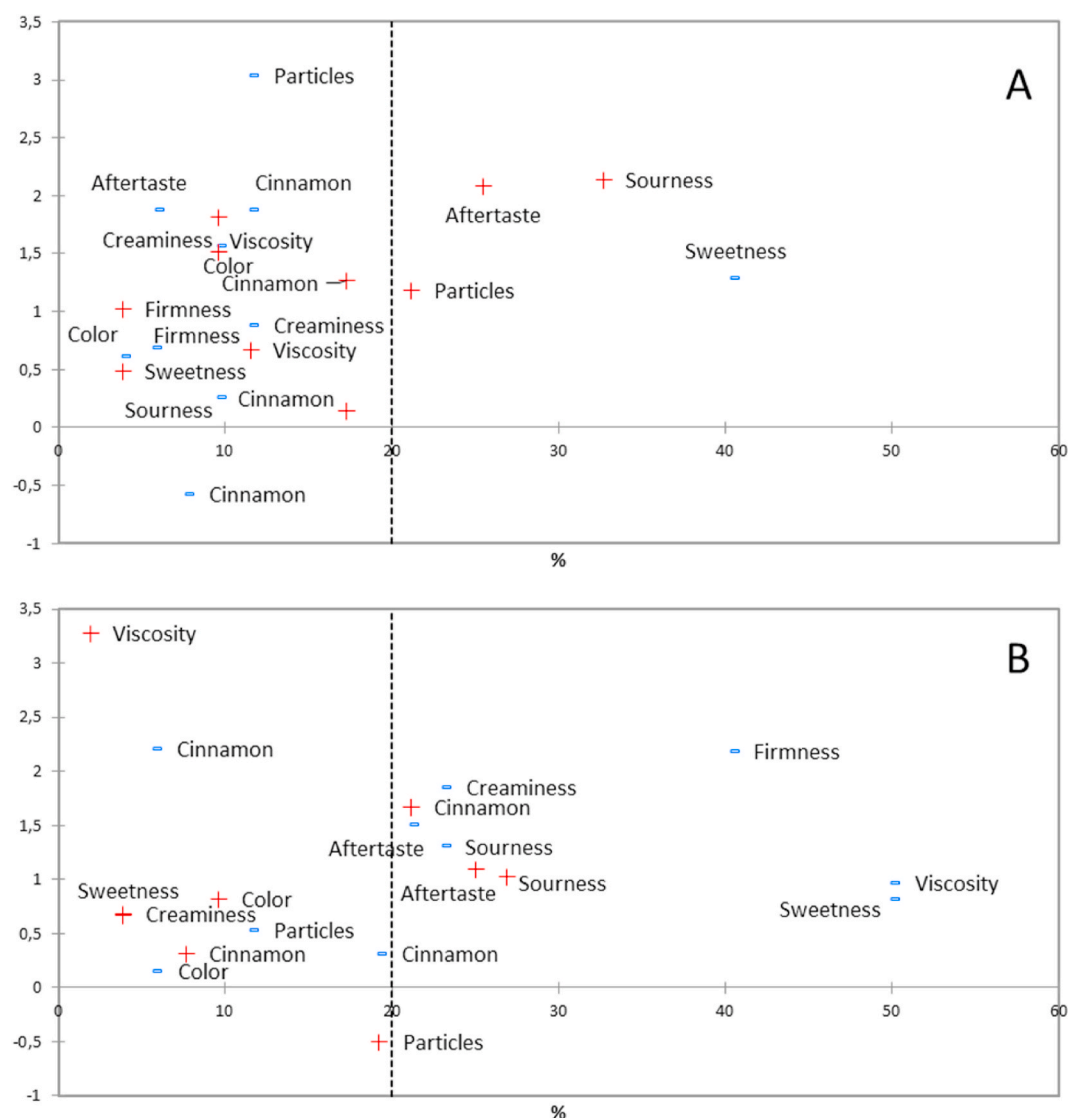


Fig. 4. Penalty analysis of selected developed yoghurts (Fig. 3A: 0.5Cs; Fig. 3B: 0.5Cy; “too low intensity” indicated with “-”, and “too high intensity” indicated with “+”).

CRedit authorship contribution statement

N. Jiménez-Redondo: Investigation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **A.E. Vargas:** Investigation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **C. Teruel-Andreu:** Investigation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **L. Lipan:** Writing – review & editing, Visualization, Supervision, All authors have read and agreed to the published version of the manuscript. **R. Muelas:** Methodology, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **F. Hernández-García:** Conceptualization, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **E. Sendra:** Conceptualization, Methodology, Visualization, Supervision, All authors have read and agreed to the published version of the manuscript. **M. Cano-Lamadrid:** Conceptualization, Methodology, Visualization, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The following authors have affiliations with organizations with indirect financial interest in the subject matter discussed in the manuscript:

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