



Production and Quality Evaluation of a Natural Yoghurt Enriched with Phenolic Extracts of *Curcuma longa*

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ABSTRACT

Background: Functional foods are currently in great demand by consumers. This study aims to assess the impact of hydroethanol extract of *C. longa* (turmeric) as a coloring agent and functional ingredient, by evaluating its effects on physicochemical and microbiological properties, as well as on the organoleptic quality of natural yoghurt-type fermented milk.

Methods: Curcuma extracts, obtained from *C. longa* rhizomes, were added at 1 mg, 2 mg, 3 mg, 4 mg and 5 mg per 100 mL of milk. A control yoghurt was also prepared without the addition of *C. longa* extract. Physico-chemical analysis was carried out to measure pH, acidity and viscosity. Microbiological analysis was carried out to assess the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus*. Finally, a sensory evaluation was carried out with 20 panellists.

Result: Physico-chemical analyses revealed that the incorporation of turmeric extract led, on the one hand, to a significant lowering of pH with an increase in acidity and an improvement in yoghurt viscosity. On the other hand, yoghurts enriched with turmeric extract showed no significant difference in sensory appearance, apart from an improved perception of color and flavor. Overall, the incorporation of extracts at doses of 1 mg to 3 mg had a favorable impact on physicochemical parameters, with sensory acceptability, a reduction in the viability of specific lactic acid bacteria to the acceptability threshold prescribed by the International Dairy Federation, controlled post-acidification and better bioprotection of yoghurt against spoilage micro-organisms at the expiration date.

Key words: Alicaments, *Curcuma longa*, Polyphenols, Yoghurt.

INTRODUCTION

Today, consumer interest has evolved considerably, driven by a constant search for new flavors and a growing awareness that has led consumers to demand organic products without chemical additives (Bulut *et al.*, 2022; Zahid *et al.*, 2023). These trends have created new dynamics in the food market, encouraging manufacturers to move away from toxic products and towards sustainable additives of natural origin. This transition is driven by the need to develop healthy foods, which have become an essential component of modern diets (Ali *et al.*, 2023; Deepa *et al.*, 2016), which are often added to fresh milk before fermentation. Various fruits, vegetables and spices, including turmeric, are incorporated into yogurt to provide natural flavors and colors.

Yoghurt is a widely consumed dairy product with many health benefits, made through the fermentation of milk. It contains living microorganisms, in particular *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* (Celik *et al.*, 2023), as well as sometimes additional probiotics and their beneficial metabolites. These are combined with the proteins, fats, minerals and vitamins naturally found in milk. However, plain yogurt lacks essential nutrients such as ascorbic acid, folic acid, iron and functional components like phenolic flavonoids and tannins (Chryssanthopoulos and Maridaki, 2009; Okur, 2022; Rashwan *et al.*, 2022).

Turmeric (*Curcuma longa* L.) is a perennial plant in the ginger family (*Zingiberaceae*) (Guerra *et al.*, 2022), native

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to Asia and the southern tropics. It is now grown in tropical and subtropical regions around the world (Jurenka, 2009).

The key ingredient in *curcuma longa* lies in its richness in phytochemicals, including curcumin, demethoxy curcumin and bisdemethoxycurcumin, as well as a high content of volatile oils (Shehzad *et al.*, 2013), making it an antioxidant, antimicrobial, anti-inflammatory, anti-cancer and anticoagulant (Ağören and Akkol, 2023; Sabir *et al.*, 2020). Particularly effective for human health compared to synthetic molecules. Therefore, it represents a major asset for the food industry, as phenolic compounds inhibit the oxidative deterioration of food ingredients, thus guaranteeing high product quality and better nutritional value.

The purpose of this study was to evaluate the use of hydro-ethanolic extract as a functional coloring additive in firm yoghurt and to observe its physicochemical and microbiological properties after 1, 7, 14 and 21 days of storage at 4°C. In addition, sensory tests were performed on the samples after one day of storage.

MATERIALS AND METHODS

Plant material

The study was carried out from September 2024 to January 2025 in the research laboratories of Nutrition and Food Sciences, Mostaganem, Algeria.

The rhizomes of *Curcuma longa* L. used in this study were purchased in the form of fresh (organic) rhizomes from herbalists in the city of Tlemcen, in northwestern Algeria. They were dried in an oven at 40°C, in the laboratory for 03 weeks until a constant weight was reached, the moisture content was estimated at 8.76%. After drying, the rhizomes were crushed using an universal mill M 20 (IKA model) to a fine turmeric powder (grain size <2 mm) and then stored in sterile jars hermetically at -20°C for later use. The preparation of the yoghurt was carried out using *Streptococcus thermophilus* (St) (ATCC 19258) and *Lactobacillus delbrueckii subsp. bulgaricus* (Lb) (ATCC 11842), both supplied by SACCO (Italy). Ethanol (e°99.6%) and chemicals were purchased from Sigma-Aldrich Co. (Germany). All reagents and chemicals used were of the highest quality.

Extraction of total polyphenols

Ultrasonic extraction was performed using the process of (Yang *et al.*, 2020), with some changes in the liquid-solid ratio. Specifically, 10 grams of *Curcuma longa* was weighed and mixed with 100 mL of 70% ethanol solvent and macerated for 1 hour at room temperature. The water surrounding the container was circulated and replaced regularly to maintain an optimal temperature. After extraction and ultrasonic treatment (VWR Cleaner, USA), the mixture was filtered through a 0.45 µm regenerated cellulose syringe filter (Phenomenex) to separate the solids from the liquids. After filtration, the sample was centrifuged at 13000 rpm for 10 minutes. The top layer was collected and stored at -20°C for purification and evaporation using a rotavapor (BUCHI R-100, Switzerland) and then frozen at -

20°C in round-bottomed vials and freeze-dried at -80°C for 72 h in a freeze dryer (Biobase, China).

Preparation of fermented yoghurt-type milk

The milk used to produce fermented milk of the firm yoghurt type is a pasteurized cow's milk with 26% solids, produced by an industrial dairy group, the GIPLAIT unit in Mostaganem, Algeria. The milk was heated to 45°C and then dispensed into sterile 100 mL containers eighteen jars. each batch containing 03 jars, they were inoculated with a starter culture composed of *S. thermophilus* and *L. bulgaricus* at a concentration of 3% in an assay ratio of 02 *S. thermophilus* to 01 *L. bulgaricus* (Khelifi *et al.*, 2018). Hydroethanolic extract of *Curcuma longa* was added to each container at doses of 0 mg (control), 1 mg, 2 mg, 3 mg, 4 mg and 5 mg per 100 mL. The containers were then closed, incubated in an incubator and left to ferment at 45°C for 4 hours, the samples were cooled to 4°C and stored for 21 days.

Measurements and controls of fermented milk

Measurements of pH, titratable acidity and viscosity and count of specific strains were carried out in triplicate on the 1st, 7th, 14th and 21st days during the post-acidification period of the fermented milk stored at 4°C. Sensory properties were evaluated after 24 hours of storage. The pH and acidity of the experimental yoghurts were assessed in accordance with the methods described by AOAC (Arioui *et al.*, 2016).

Dornic acidity

It was determined by titration of 10 mL of yoghurt, with the addition of a few drops of phenolphthalein as a colored indicator, using a solution of soda (sodium hydroxide) 1/9 N and expressed in degrees Dornic (°D).

The pH

It was measured using a METTLER TOLEDO New Five Easy laboratory pH meter (Italy), previously calibrated with two buffer solutions, one acidic and the other basic.

Viscosity measurement

The viscosity was measured using a type C viscometer (German DIN 53015). The principle is based on the fall of a HAAKE FALLING BALL model by (Shehata *et al.*, 2016) and the dynamic viscosity was deduced by applying the following formulas:

$$\mu = K. (\xi_{\text{ball}} - \xi_{\text{yoghurt}}). L$$

$$K = 2r^2 g / 9.x$$

μ = Dynamic viscosity (Kg·m⁻¹·s⁻¹).

K = Constant where $K = 6.59 \times 10^{-4}$.

r = Bale radius, $r = D/2 = 0.55$ cm.

x = Bale flow distance where $x = 10$ cm.

g = Gravitational force, $g = 9.81$ m/s².

ξ_{ball} = Bale density where $\xi_{\text{ball}} = 1.68 \times 10^4$ Kg·m⁻³.

ξ_{yoghurt} = Density of yoghurt where $\xi_{\text{yogurt}} = 10.70 \times 10^2$ Kg·m⁻³.

t = Time elapsed between the two viscometer points A and B. The measurement was repeated 03 times for each control performed.

Microbiological analyses

One g of yoghurt sample was weighed and transferred to a sterilized jar and then 9 mL of sterile physiological solution (0.9% NaCl). This mother suspension was in turn used to prepare a series of further dilutions. Required dilution was spread on M17 agar (Condalab, Spain) and incubated at 37°C for 48 hours. The Probiotic count was carried out on MRS agar (Condalab, Spain), enriched with L-cysteine hydrochloride (0.5 g/L), with incubation at 37°C for 48 hours under anaerobic to facultative aerobic conditions (Arab *et al.*, 2022). Only plates containing between 30 and 300 colonies were retained for analysis. Results were expressed as log colony forming units per milliliter (log CFU/g).

Organoleptic test

In order to evaluate the sensory properties of yoghurts with or without *Curcuma longa* extracts, an acceptance analysis was carried out using a 10-point hedonic scale, evaluated by 20 untrained panelists aged 22 to 55 years among the staff (teachers, students and laboratory engineers) affiliated with the Department of Agronomy of the University of Mostaganem, in Algeria. The attributes assessed were color, flavor, texture, aroma and overall acceptability. Samples were given to panelists in 25 mL plastic cups labelled

with a three-digit code. The panelists cleansed their palates with water and unsalted bread between each sample.

Statistical analysis

Parametric results from three replicated trials were statistically analyzed using a fully randomized bidirectional analysis of variance (ANOVA) with a comparison of means using the Newman-Keuls test. The results of the organoleptic assay were processed using the nonparametric Friedman assay. All calculations were performed using the Stat Box 6 software. The effects of the factors were assessed at two probability thresholds, $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Evaluation of the physicochemical parameters of yoghurt (pH, acidity and viscosity)

Yoghurt is a dairy product obtained by fermenting milk by starter cultures. Its quality is mainly assessed by measuring pH and titratable acidity and viscosity, which are key indicators. The results of the analysis of physicochemical parameters are presented in (Table 1).

Titrateable acidity and pH

The titrateable acidity and pH are complementary characteristics in yoghurt, as the decrease in pH values

Table 1: pH, acidity and viscosity of yogurt taken with and without *Curcuma longa* extract during storage.

Samples	Storage (days)	pH	Titrateable acidity (% by weight of lactic acid)	Dynamic viscosity
0 %	1	4.61 ^a ±0.01	80 ^c ±1	693 ^a ±6.46
	7	4.36 ^a ±0.02	90.33 ^c ±2.082	746 ^a ±6.46
	14	4.47 ^d ±0.031	88.67 ^d ±1.528	735 ^a ±5
	21	4.2 ^a ±0.017	90 ^d ±1	680.33 ^a ±7.76
1 %	1	4.59 ^a ±0.006	82,67 ^b ±0.577	672.33 ^b ±2.517
	7	4.34 ^a ±0.025	92 ^c ±2	725 ^b ±5
	14	4.55 ^a ±0.01	91,67 ^c ±1,528	722.67 ^b ±10.06
	21	4.24 ^a ±0.02	91 ^c ±1	661 ^b ±7.94
2 %	1	4.56 ^b ±0.01	84 ^b ±1	666 ^c ±2
	7	4.36 ^a ±0.02	94 ^b ± 2,646	714 ^b ±2
	14	4.52 ^b ±0.01	92.67 ^c ±0.577	716.33 ^c ±5.86
	21	4.2 ^b ±0.02	93 ^{cd} ±1	675.67 ^a ±3.51
	1	4.53 ^c ±0.01	86 ^a ±1	659.67 ^d ±1.53
3 %	7	4.31 ^{ab} ±0.031	93 ^c ±1	715.67 ^b ±5.68
	14	4.47 ^c ±0.01	94 ^{bc} ±1	705.33 ^d ±3.51
	21	4.19 ^b ±0.02	96 ^b ±1	664.67 ^b ±4.93
	1	4.48 ^d ±0.015	82 ^b ± 1	655.67 ^e ±1.53
	7	4.30 ^{ab} ±0.006	97,33 ^b ±1.528	707 ^b ±4
4 %	14	4.39 ^d ±0.015	96 ^b ±1	704.33 ^e ±6.86
	21	4.17 ^b ±0.01	98 ^b ±1	644.67 ^c ±2.52
	1	4.44 ^e ±0.02	86,33 ^a ±1.528	650 ^f ±2
	7	4.27 ^b ±0.026	105 ^a ±2	696.33 ^c ±5.66
5 %	14	4.34 ^e ±0.01	99 ^a ±1	685 ^c ±2
	21	4.12 ^c ±0.02	103 ^a ±2	638.67 ^c ±6.11

Physicochemical parameters of experimental yoghurts. The data are expressed as mean values, followed by corresponding standard deviations, with a number of replicates $n = 3n = 3n = 3$. The letters a, b, c, d, e, etc., indicate pairwise comparisons of means using the Newman-Keuls test.

corresponds to the transformation of lactose into lactic acid (Wang *et al.*, 2020; Dahou *et al.*, 2024). Based on pH values obtained from different yogurt samples fortified or not with varying doses of turmeric extract, a very significant decrease ($p \leq 0.01$) was recorded during the post-acidification period. The pH values recorded from day 1 to day 21 of storage were around 4.61 and 4.12 respectively. Conversely, acidity increased very significantly ($p \leq 0.1$) and proportional to the storage time and with increasing doses of *C. longa*, showing an increase from 80 to 103°D from day 1 to day 21. This phenomenon follows the action of lactic acid cultures that break down lactose into lactic acid. Specifically, the *Streptococcus thermophilus* uses the free amino acids present in milk or those resulting from the hydrolysis of casein by the *Lactobacillus bulgaricus* filter (Yamauchi *et al.*, 2019). Anaerobic conditions are created due to the release of CO₂ as well as the synthesis of formic acid, a growth factor necessary for *Lactobacillus bulgaricus*. The acidity recorded in this range remains within the standards required for yoghurts fit for human consumption ($\leq 115^\circ\text{D}$), according to (Aktypis *et al.*, 2023) the same results were recorded by (Guemidi *et al.*, 2024) who reported a significant decrease in pH ($p \leq 0.01$) and increased acidity in yogurts fortified with hydroethanolic peppermint extract.

Viscosity

The viscosity of products enriched with different doses extracted from *Curcuma longa* was relatively lower than that of the control sample without phenolic extract. In addition, the values recorded in experimental samples tended to increase up to 21st days of cold storage, reaching average values of about 675.7 ± 3.5 . According to Schmidt and Walter (1994), the viscosity obtained is probably due to the metabolic compound, called Exopolysaccharides (EPS), produced by *Streptococcus thermophilus*, which is

composed of carbohydrates such as β -glucan and β -fructan, which can bind to milk caseins, thus increasing yoghurt viscosity (Khelifi *et al.*, 2018). The results of the viscosity tests showed a significant decrease ($p \leq 0.05$) in viscosity with an increase in the incorporation rates of turmeric extracts from 0 to 5 mg in the fermented milk samples. This is because the addition of turmeric extracts destabilizes lactic coagulation and weakens the aggregation of insoluble proteins in yoghurt. Similar results were reported by (Salva *et al.*, 2022) in a study of a soy and quinoa-based yoghurt.

Counting yoghurt cultures

The results of the enumeration of the lactic acid bacteria *Streptococcus-thermophilus* and *Lactobacillus bulgaricus* in yoghurt samples enriched with phenolic extracts of *Curcuma longa* are shown in Fig 1 and 2. The study showed that as the incorporation levels of the hydroethanolic extracts of *Curcuma longa* increase, the number of bacteria (*St. thermophilus* and *Lb. bulgaricus*) decreased significantly in the experimental yoghurts. When a high concentration of *C. longa* (4 mg% and 5 mg%) was added to the fermented milk, a noticeable reduction is observed. In the same context, studies by (Yang *et al.*, 2020) confirmed that *C. longa* extract triggered anti-bacterial activity at a dosage of over 3.5 mg%.

Effect on lactic acid bacteria the number of *St. thermophilus* in fermented milk with the added hydro extract of *C. longa* increased from 362×10^6 CUF/mL to 383×10^6 CUF/mL on average from day 1 to day 14 at 4°C, followed by a decrease in bacterial growth on day 21 of storage. Besides, the number of *S. thermophilus* increased non-significantly ($p \geq 0.01$) with increasing extract concentrations from 1mg to 3 mg and decreases significantly with higher doses of *C. longa* from 383×10^6 CFU/mL to 182×10^6 CFU/mL with doses of 4 mg and 5%. As for *L. bulgaricus subsp. delbrueckii* in yoghurts with hydroethanolic extract, their number decreases

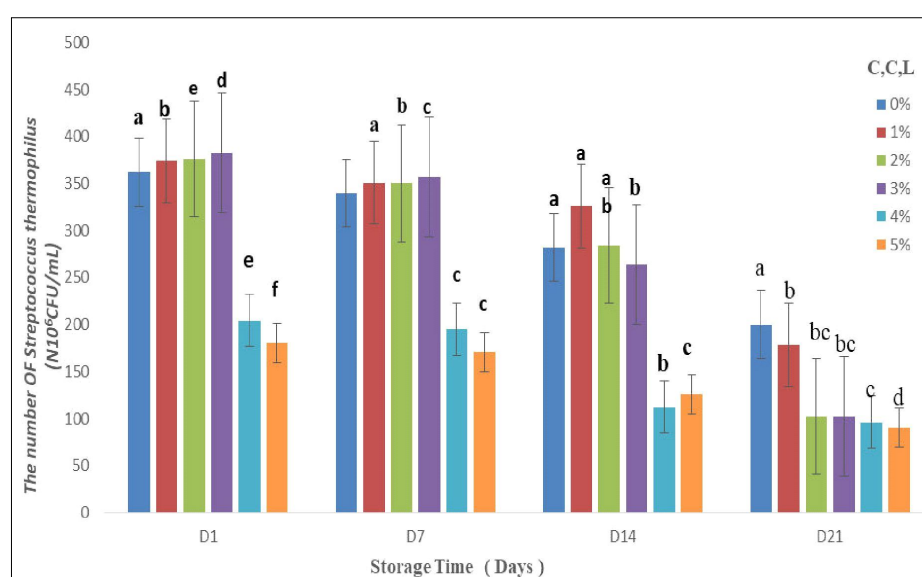


Fig 1: Evaluation of the number of *Streptococcus thermophilus* ($\times 10^6$ CFU/mL) in yogurt with and without turmeric extract.

from 362×10^6 to 280×10^6 CFU on average from day 1 to day 14 to 21 day, then decreases further to 200×10^6 CFU on day 21. In the control group, a decrease related to storage time is observed and a gradual decrease is also noted with decreases from 374×10^6 to 178×10^6 CFU/mL.

Very significant decreases ($p \leq 0.01$) in the number of *L. bulgaricus* were observed with an increase in hydro-ethanolic extract levels of 0 to 5 mg in fermented milk during post-acidification. Similar results are reported in studies conducted by various authors on the effect of incorporating polyphenol extracts on yogurt cultures. According to (Okur, 2022), the incorporation of polyphenols extracted from tea in low doses during milk fermentation improves growth *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

and according to (Mediza Romero *et al.*, 2021), reported that adding blackcurrant phenolic extracts before yogurt fermentation stimulates the growth of *St. thermophilus* and *Lb. bulgaricus* due to the high methoxyl content in blackcurrant extracts. This discovery supports the idea of growth-promoting agents stimulating lactic acid strains of yoghurt. In addition, polyphenols have prebiotic effects on the modulation of the gut microbiota. Several studies *in vitro* have shown that certain polyphenols (Lorusso *et al.*, 2018) can modify the composition of the gut microbiota, inhibiting some bacteria while promoting others (Dueñas *et al.*, 2015). Shown that the phenolic compounds in grape seed extracts significantly increase the growth of *Bifidobacterium* probiotics in the gastrointestinal tract,

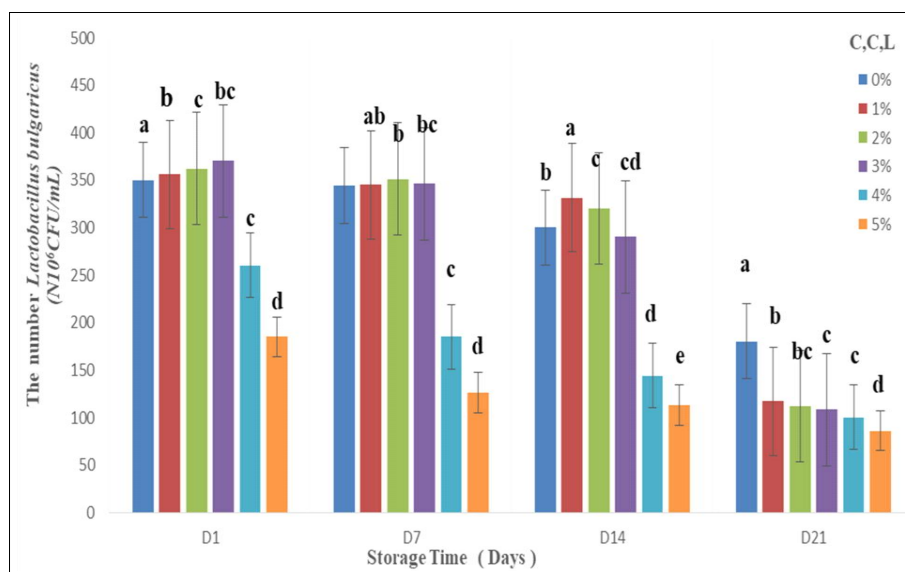


Fig 2: Evaluation of *Lactobacillus bulgaricus* ($\times 10^6$ CFU/mL) in yogurt with and without turmeric extract.

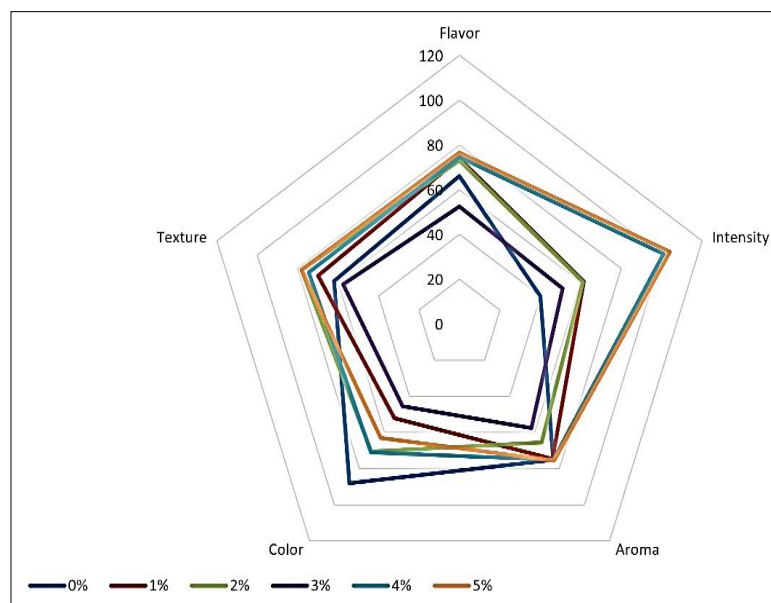


Fig 3: Sensory scores of yogurt with and without turmeric extract (n=20).

while the growth of *Lb.bulgaricus* is not affected. According to (Melini and Melini, 2021), Phenolic compounds do not affect the viability of probiotic bacteria. These results are in line with those of our study, in which we observed that lactic acid bacteria in yoghurt, *Lactobacillus delbrueckii subsp. bulgaricus* and *S. thermophilus*, showed better growth in a medium enriched with polyphenol extracts of turmeric.

Organoleptic test

Initially, 30 panelists participated in the sensory analysis of yoghurts. However, due to errors in data collection, such as missing personal information or incomplete responses, the results shown below in Fig 3 correspond to 20 participants (11 males and 9 females) aged 21 to 54 years. According to the results of the sensory evaluation test of yoghurt with turmeric extract, the addition of the extract did not alter the texture of the yoghurt. The color, smell and taste evaluation showed that products at 3% and 4% were the most preferred. These results are associated with the bitter and spicy taste of turmeric and the rhizome has an aroma due to the presence of ar-turmerone (Silva *et al.*, 2005). This characteristic is also seen with turmeric in other products, such as bread, where wheat flour is replaced with turmeric powder in Korean jellies (Guerra *et al.*, 2022). It is essential to establish that plant-based additives tend to impart specific flavors and aromas due to the volatile compounds present in the raw materials.

CONCLUSION

The use of additives in the food industry is a challenge, especially when it comes to replacing them with beneficial substances, such as natural colorings. Enriching yoghurt with *Curcuma longa* extracts increases its content of phenolic compounds, thus enhancing its functional character. In addition, the incorporation of this extract does not significantly change the physicochemical properties of the yoghurt, although at high concentrations some alterations may be observed. In addition, turmeric contributes to the improvement of the microbiological properties of the product by promoting the viability of lactic ferments up to 21 days of storage. Based on sensory evaluations, the panelists recommended an optimal concentration of 3 mg of turmeric extract per 100 mL of milk in the yoghurt.

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Disclaimers

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Conflict of interest

The authors declare no conflict of interest. The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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