



# Evaluation of instrumental and sensory measurements using multivariate analysis in probiotic yogurt enriched with almond milk

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## Highlights

- Consumers demand the purchase of fortified dairy products.
- Instrumental (color, texture) and sensorial attributes are critical tests for novel food.
- Almond milk has high nutritional value with unique textural and sensorial properties.
- Almond milk is an innovative and attractive additive in probiotic yogurt.

**Abstract** In this study, the effect of almond milk addition on color, texture and sensory attributes of probiotic yogurt was investigated. The data generated in combination with instrumental (color and texture parameters) and sensory measurements was analysed statistically to describe a product's attributes scientifically. Statistical analysis illustrated that almond milk rate and storage time had a significant ( $P < 0.05$ ) effect on the color and textural parameters of yogurt. Compared with the sensorial parameters, generally there were statistically significant differences among samples, whereas insignificant effect was determined among storage days. The results of descriptive statistics (Principle Component and Hierarchical Cluster Analysis) indicated that the relationships among the analysed attributes were determined. In addition, statistical data has demonstrated that almond milk may be used as a novel and functional ingredient in both industrial

and research areas for development, innovation, quality, and safety of dairy products.

**Keywords** Probiotic yogurt · Almond milk · Instrumental and sensory measurements · Multivariate analysis

## Introduction

Yogurt, a fermented dairy product, has nutritional value, therapeutic benefits, and a broad popularity in the dairy market. Yogurt is an important source of high quality proteins and micronutrients such as calcium. Due to its high digestibility and bioavailability of nutrients, yogurt could contribute to meet the daily intake of various nutrients. There is an increasing interest in the production and consumption of yogurt including probiotics. Probiotic yogurt including *Bifidobacterium* and *Lactobacillus* subsp. has also been proven to provide beneficial effects and is used extensively in the dairy industry as a nutrient-dense food. Even, recent studies have focused that consumption of probiotic foods such as dairy products may be one of the promising therapeutic approaches for the prevention or treatment of new pathogenic viruses such as SARS-CoV-2 (COVID-19) (Lang et al. 2011; Fonseca et al. 2020). Nowadays, probiotic yogurt has been manufactured by means of enrichment and fortification with plant-based ingredients to improve multifunctional benefits. Consumers have demanded also the purchase and use of fortified dairy products, especially vegetable milk-based products. The vegetable milks can be also used to improve nutritive value a variety of products such as yoghurt, ice-cream, cheese and butter in order to respond to health conscious consumers interested in the nutritional and health-promoting benefits (Mäkinen et al. 2016; Jeske et al. 2018; Silva et al.

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2020). Almond milk could be mixed with other milks for yogurt production. Almond milk contains high quality protein, carbohydrates with low glycemic index, unsaturated fatty acids, vitamins, minerals, dietary fibre, phenolic acids and flavonoids, all of which are essential nutrients for daily consumption and might be stimulator nutrients for probiotic bacteria (Hasan 2012; Sethi et al. 2016; Sousa and Kopf-Bolanz 2017; Kundu et al. 2018). Almond milk displays unique textural and sensorial properties with high industrial yield and dry matter contents (Yilmaz-Ersan and Kurdal 2014; Chalupa-Krebzdak et al. 2018; Holscher et al. 2018; Franklin and Mitchell 2019; Yilmaz Ersan and Topcuoglu 2019).

Critical tests for the development of enriched yogurt are to assess the effect of varying supplements (fruits, vegetables, nuts, plant based milk etc.) and manufacturing processes (starter type/ratio, heat treatment, incubation/storage conditions) on yogurt, to compare with similar products, to improve the demand of existing product, to create attractive product and to improve its sensorial acceptability, and instrumental parameters such as color and texture. However, geographical differences, natural content, ethical concerns, and health existing are important factors in determining consumers' preference for color features, textural characteristics and sensorial perception (Ozcan 2013; Singham et al. 2015; Costa et al. 2017; Yilmaz-Ersan et al. 2017; Amaral et al. 2020).

The sensory analysis of yogurt is based on the human senses, expensive and tedious, thus, researchers and manufacturers have focused on decreasing time and money spent and increasing reproducibility by removing the physiological and psychological variation associated with panelists. Multivariate analysis techniques (Pearson's correlation coefficient, principal component analysis and hierarchical analysis) between these instrumental measurements (color, texture) and sensory evaluation show how more controlled instrumental tests are related to perceived sensory properties of yogurt. Non and/or linear correlations may help in predicting sensory perception which can be used in changing supplements and manufacturing process as per the requirements (Drake et al. 1999; Leiva and Figueroa 2010; Ghosh and Chattopadhyay 2012; Phuong et al. 2017; Dias et al. 2020; Pinto et al. 2020).

Recently, the global COVID-19 pandemic crisis once again revealed the importance functional foods fortified with probiotics, prebiotics, vitamins (e.g. C, D, E and A) and antioxidants. Thus, food sector experts and academia have focused on developing novel healthy products or renewing current products by improving their health benefits (Galanakis 2020; Galanakis et al. 2020; Rizou et al. 2020). While a lot of research has primarily focused on the chemical composition and nutritious value of the yogurt

with enriched different plant based supplements (Kolapo and Olubamiwa 2012; Bansal et al. 2016; Ozturkoglu-Budak et al. 2016; Ozcan et al. 2017; Demirkesen et al. 2018; Yildiz and Ozcan 2019), some reported the possible physicochemical similarities and differences among types. However, there has been a few multivariate analysis techniques on yogurt using the range of available instrumental techniques and sensorial properties. Therefore, the objectives of this study were (i) to evaluate color, textural and sensorial changes in probiotic yogurt enriched with almond milk (ii) to correlate instrumental and sensory analysis measurements of probiotic yogurt.

## Material and methods

### Materials

For manufacturing of probiotic yogurt samples, skim milk powder was purchased from a local dairy factory (EKER Dairy Co., Bursa, Turkiye). Pasteurized commercial almond milk was obtained from Kocamaar Farm Co., (Mugla, Turkiye). Probiotic yogurts were prepared by using the freeze dried strain mix composed of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* (Yo-Mix 205 LYO 250 DCU; Danisco, France).

### Starter culture and inoculum preparation

The culture was propagated in 12 g 100 mL<sup>-1</sup> reconstituted sterile non-fat milk (autoclaved at 121 °C for 15 min). The probiotic mix was activated in sterile reconstituted skim milk and incubating at 40 °C, until a final pH of 4.8 was achieved.

### Production of probiotic yoghurt with enriched almond milk

Probiotic yogurt samples were manufactured at the Food Pilot Plant of Bursa Uludag University-Food Engineering Department (Bursa, Turkiye). Skim milk powder was reconstituted in distilled water at 10.70% (w/v) to yield reconstituted skim milk of the same overall composition as raw skim milk. Reconstituted skim milk was heated to 90 °C for 10 min in a water bath. Five proportions of reconstituted skim milk to almond milk (100:0, 75:25, 50:50, 25:75, 0:100) were prepared by mixing the heated reconstituted milk with the almond milk before cooling to the inoculation temperature. Probiotic yoghurt samples were identified as RM<sub>(100)</sub> (including 100% reconstituted milk), AM<sub>(100)</sub> (including 100% almond milk), RAM<sub>(75:25)</sub>

(including 75% reconstituted milk and 25% almond milk), RAM<sub>(50:50)</sub> (including 50% reconstituted milk and 50% almond milk) and RAM<sub>(25:75)</sub> (including 25% reconstituted milk and 75% almond milk). Each experimental mixture was inoculated with a 3% mixed culture (*Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, *Lb. acidophilus* and *B. animalis* subsp. *lactis*). The inoculum levels of each bacterium were determined to give a final concentration of approximately  $8\text{--}9 \log_{10}$  CFU mL<sup>-1</sup> in milk and then incubated at 40 °C until the final pH of 4.6 was achieved. At the end of the incubation period, the samples were kept at room temperature ( $20 \pm 1$  °C) for 30 min and then transferred to a refrigerator at  $4 \pm 1$  °C for 21 days.

### Instrumental color analysis

Instrumental color analysis of yogurt samples was performed by using a Minolta Chromameter (Konica Minolta Co., Ltd., Osaka, Japan) calibrated with white calibration plate as specified by the manufacturer. CIELab color scale was used to obtain L\*, a\* and b\* values. In the CIELab color scale, the L\* parameter ranges from 0 to 100, indicating the color variation (brightness) from black to white; the \* axis shows the variation from red (+ a\*) to green (− a\*); and the b\* axis shows the variation from yellow (+ b\*) to blue (− b\*). Chromaticity (C\*), which measures the color intensity, was calculated by  $C^* = (a^{*2} + b^{*2})^{1/2}$ . The hue angle (H\*) was calculated by  $(H) = \arctan(b^*/a^*)$ . Color differences ( $\Delta E^*$ ), among samples, were calculated according to the equation formula (Bernat et al. 2015):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

### Instrumental texture analysis

Textural properties of yogurt samples were evaluated instrumentally using a texture analyzer TA-XT Plus (Stable Micro System Ltd, Model TA-XT plus, Surrey, UK), as described in Yilmaz-Ersan et al. (2017). Before textural analysis, the samples were left at room temperature (25 °C). Textural attributes were determined by back extrusion method fitted with a 5 kg load cell. A 40-mm-diameter cylinder probe was used to measure the texture profile of yogurt and penetrated the samples to 75% of their original depth. The speed of the probe was fixed at 0.1 mm/s during the pretest compression and relaxation of the samples. The distance of penetration from the surface of sample was set at 20 mm. Basic textural parameters like firmness, consistency, cohesiveness and viscosity indexes were calculated using the Texture Expert Exceed software (v 2.55) extracted from the resulting force time curves. All measurements were carried out in triplicates.

### Sensory analysis

Sensory evaluation of samples was conducted by a panel of seven trained panelists, fulfilling the requirements for sensory sensitivity according to the requirements of ISO standards. Each panelist was selected on the basis of interest and experience in sensory evaluation of dairy products from the Bursa Uludag University. Yogurts were removed from a refrigerator (4 °C) 1 h prior to sensory evaluation, and kept at room temperature ( $22 \pm 2$  °C). All samples were presented in three digit blinding codes and served simultaneously with a glass of water for mouth rinsing among samples. A standard five-point hedonic scale from 1 (the lowest grade) to 5 (the highest grade) was used to measure acceptance-preference of product. All the samples were evaluated for sensory attributes such as color, appearance (syneresis), structure/body, texture, odor, taste (type and desirability), sourness and overall acceptability.

### Statistical analysis

The results were statistically evaluated using analysis of variance (ANOVA) and Fisher's least significant difference (LSDs) method at a significance level of 0.05 (Minitab 17, USA). The correlation between the instrumental measurements and sensory properties was determined using the Pearson Correlation Coefficient (r). In order to analyse the relationship between different variables and probiotic yogurt samples based on the individual response categories, Principle Component Analysis (PCA) with varimax rotation was performed on individual data using Statistica software. Hierarchical Cluster Analysis (HCA; clustering) was conducted in order to identify different groups of products. The Ward Method, with square Euclidean distance, was used to determine the preliminary number of clusters. (Statistica software Version 10.0 StatSoft Inc, France).

### Results and discussion

The colour of yogurt is an important indicator which affects quality, freshness, flavour expectation, commercial value and acceptability of product and is directly influenced by the milk types used in the formulation. Yogurt should have bright white color due to milk fat, protein content and natural pigments that are present at different levels in various milks (Dufossé and Galaup 2009; Šcibisz et al. 2019). Results of instrumental color analysis were shown in Table 1. Statistical analysis illustrated that almond milk rate and storage time had a significant ( $P < 0.05$ ) effect on the colour parameters. As almond milk rate of yogurt samples increased, L\*, a\* and b\* values

**Table 1** The color values of probiotic yoghurt samples

Color parameters	Treatments	Storage period (day)			
		1	7	14	21
L*	RM <sub>(100)</sub>	77.10 <sup>aB</sup>	76.53 <sup>aB</sup>	77.12 <sup>aB</sup>	81.19 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	75.32 <sup>aB</sup>	75.99 <sup>bB</sup>	76.29 <sup>aB</sup>	81.03 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	71.59 <sup>bC</sup>	74.18 <sup>bB</sup>	74.56 <sup>bB</sup>	79.42 <sup>abA</sup>
	RAM <sub>(25:75)</sub>	72.44 <sup>bB</sup>	72.36 <sup>cB</sup>	72.48 <sup>cB</sup>	77.53 <sup>bA</sup>
	AM <sub>(100)</sub>	62.82 <sup>cB</sup>	64.07 <sup>dB</sup>	64.99 <sup>dB</sup>	69.43 <sup>cA</sup>
a*	RM <sub>(100)</sub>	− 3.01 <sup>aA</sup>	− 2.99 <sup>aA</sup>	− 3.11 <sup>aAB</sup>	− 3.28 <sup>aAB</sup>
	RAM <sub>(75:25)</sub>	− 2.68 <sup>bAB</sup>	− 2.67 <sup>bAB</sup>	− 2.70 <sup>bA</sup>	− 2.83 <sup>bA</sup>
	RAM <sub>(50:50)</sub>	− 2.40 <sup>cA</sup>	− 2.16 <sup>cA</sup>	− 2.18 <sup>cA</sup>	− 2.26 <sup>cA</sup>
	RAM <sub>(25:75)</sub>	− 1.65 <sup>dAB</sup>	− 1.59 <sup>dAB</sup>	− 1.73 <sup>dA</sup>	− 1.74 <sup>dA</sup>
	AM <sub>(100)</sub>	− 0.85 <sup>eA</sup>	− 0.44 <sup>eB</sup>	− 0.43 <sup>eB</sup>	− 0.42 <sup>eB</sup>
b*	RM <sub>(100)</sub>	6.91 <sup>aB</sup>	7.10 <sup>aB</sup>	7.09 <sup>aB</sup>	7.71 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	6.25 <sup>bB</sup>	6.19 <sup>bB</sup>	6.46 <sup>bB</sup>	6.92 <sup>bA</sup>
	RAM <sub>(50:50)</sub>	5.33 <sup>cC</sup>	5.55 <sup>cB</sup>	5.71 <sup>cB</sup>	6.07 <sup>cA</sup>
	RAM <sub>(25:75)</sub>	4.20 <sup>dB</sup>	4.28 <sup>dB</sup>	4.41 <sup>dB</sup>	4.72 <sup>dA</sup>
	AM <sub>(100)</sub>	5.40 <sup>cA</sup>	5.61 <sup>bcA</sup>	5.89 <sup>cA</sup>	5.74 <sup>cA</sup>
H*	RM <sub>(100)</sub>	293.55 <sup>aA</sup>	292.83 <sup>aA</sup>	293.71 <sup>aA</sup>	293.02 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	293.17 <sup>aA</sup>	293.30 <sup>aA</sup>	292.67 <sup>abA</sup>	292.22 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	294.17 <sup>aA</sup>	291.31 <sup>bB</sup>	290.95 <sup>cB</sup>	290.41 <sup>bB</sup>
	RAM <sub>(25:75)</sub>	291.47 <sup>bA</sup>	290.38 <sup>bA</sup>	291.48 <sup>bcA</sup>	290.25 <sup>bA</sup>
	AM <sub>(100)</sub>	278.93 <sup>cA</sup>	274.50 <sup>cB</sup>	274.18 <sup>dB</sup>	274.17 <sup>cB</sup>
C*	RM <sub>(100)</sub>	7.53 <sup>aB</sup>	7.71 <sup>aB</sup>	7.74 <sup>aB</sup>	8.38 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	6.80 <sup>bB</sup>	6.74 <sup>bB</sup>	6.99 <sup>bB</sup>	7.48 <sup>bA</sup>
	RAM <sub>(50:50)</sub>	5.85 <sup>cC</sup>	5.95 <sup>cBC</sup>	6.11 <sup>cB</sup>	6.48 <sup>cA</sup>
	RAM <sub>(25:75)</sub>	4.52 <sup>dB</sup>	4.57 <sup>dB</sup>	4.74 <sup>dB</sup>	5.03 <sup>eA</sup>
	AM <sub>(100)</sub>	5.47 <sup>cA</sup>	5.63 <sup>cA</sup>	5.91 <sup>cA</sup>	5.76 <sup>dA</sup>
ΔE*	RM <sub>(100)</sub>	–	–	–	–
	RAM <sub>(75:25)</sub>	1.95 <sup>cA</sup>	1.11 <sup>dB</sup>	1.16 <sup>dB</sup>	1.03 <sup>cB</sup>
	RAM <sub>(50:50)</sub>	5.78 <sup>bA</sup>	2.94 <sup>cB</sup>	3.06 <sup>cB</sup>	2.63 <sup>bcB</sup>
	RAM <sub>(25:75)</sub>	5.56 <sup>bA</sup>	5.24 <sup>bAB</sup>	5.54 <sup>bA</sup>	4.98 <sup>bB</sup>
	AM <sub>(100)</sub>	14.53 <sup>aA</sup>	12.82 <sup>aB</sup>	12.48 <sup>aB</sup>	12.28 <sup>aB</sup>

<sup>a–d</sup>Different lowercase supercripts in the same column depict the significant difference between means for samples ( $P < 0.05$ )

<sup>A–D</sup>Different uppercase supercripts in the same row depict the significant difference between means for storage time ( $P < 0.05$ )

of samples decreased. The  $a^*$  values, with all measurements minus, confirm that the greentone is dominating over the red in all samples. The  $b^*$  values, with all measurements above zero, confirm that the yellow coloration is dominating over the blue in all samples. That lightness, yellowness and greenness decreases can be explained by the decrease of the low dry matter of almond milk. Reconstituted milk used in this study contains 12% dry matter, whereas dry matter content of almond milk was 3.369%. During storage time,  $L^*$ ,  $a^*$  and  $b^*$  values of all samples increased, except for  $a^*$  values of RM<sub>(100)</sub> and AM<sub>(100)</sub>. The decrease  $a^*$  values of AM<sub>(100)</sub> may be due to the

degradation of the long chemical structure of carotenoids during storage. Significantly higher  $H^*$  values for probiotic yogurt that was produced with increasing reconstituted milk, indicate that these products tend to have more expressed yellow tone while having significantly lower  $H^*$  values for almond milk yogurt, this indicates that this product tends to have more expressed red tone. The  $\Delta E^*$  differences were categorized as imperceptible differences (0–0.5), slight differences (0.5–1.5), just noticeable differences (1.5–3.0), marked differences (3.0–6.0), extremely marked differences (6.0–12.0) and colors of different shades (above 12.0) (Kim et al. 2002). According to  $\Delta E^*$

values calculated in this study,  $RAM_{(75:25)}$ ,  $RAM_{(50:50)}$ ,  $RAM_{(25:75)}$  and  $AM_{(100)}$  samples were classified respectively as slight differences (0.5–1.5), just noticeable differences (1.5–3.0), marked differences (3.0–6.0) and colors of different shades (above 12.0). These parameters were affected by the storage time at 4 °C until 21 storage days, while  $C^*$  and  $H^*$  generally increased, the total colour difference ( $\Delta E^*$ ) decreased ( $P < 0.05$ ). Bernat et al. (2015) determined that non-dairy probiotic fermented product based on almond milk and inulin had 87.83–90.51  $L^*$  values, 5.33–5.80  $C^*$  values and 97–100.56  $H^*$  values. Whiteness in fluid milk results from the presence of colloidal particles, such as milk fat globules and casein micelles, that are capable of scattering light in the visible spectrum. The differences observed in yogurt samples seem to demonstrate the presence of an interaction between the protein source and the pigment in milk types. The carotenoids especially, can influence the milk's color, and consequently, the color of yogurt. Since reconstituted milk contains less of the yellow pigment carotene, it appears whiter than almond milk. The difference of colour parameters in samples can be explained by the effect of pigments such as carotenoids, and flavonoids, which are responsible for the red, yellow, and orange colours in dairy products such as fortified vegetable milks (Yilmaz-Ersan et al. 2017; Chudy et al. 2020).

Since consumers prefer firmer, more consistent, viscous and cohesive yogurts, textural parameters are a critical factor for determining yogurt quality (Ozcan 2013; Prajapati et al. 2016; Yilmaz-Ersan et al. 2017). Texture profile analysis is a useful technique to evaluate the firmness, consistency, cohesiveness, and index of viscosity in yogurt. Results of texture analysis were shown in Table 2. There were significant differences ( $P < 0.05$ ) within the values of textural parameters throughout storage. Firmness, the force necessary to attain a given deformation, is important to determine yogurt quality, the higher value means the firmer sample. There were significant differences in milk type and storage time for firmness values ( $P < 0.05$ ). Generally, the highest firmness values were detected in  $RM_{(100)}$  yogurt (with 100% reconstituted milk). In comparison with the sample, as the almond milk rate of samples increased, the firmness values of samples decreased. Throughout storage there were different tendencies in the level of firmness value, depending on the almond milk rate used. Yogurt with a higher ratio of reconstituted milk had a higher firmness than almond milk yogurts due to its higher protein and total solids content, of which higher protein content would cause an increase degree of cross-linkage of the gel network and result in a much denser and firmer gel structure. Bernat et al. (2015) reported that fermented almond milk including *Lb. reuteri* ATCC 55730 and *S. thermophilus* CECT 986 had a weak

gel structure due to the action of proteins, which was able to retain part of the serum present in the almond milk. Ozturkoglu-Budak et al. (2016) mentioned that firmness of control yogurt was higher than almond-fortified yogurt. The consistency serves as a viscosity guide of yogurt, higher values indicate a more viscous or thicker sample. The consistency values increased notably in samples except for  $AM_{(100)}$  yogurt (with 100% almond milk) during storage ( $P < 0.01$ ). Maximum levels of the consistency values were observed on the 14th day of storage for  $RM_{(100)}$  and  $RAM_{(25:75)}$  samples, on the 21st day of storage for  $RAM_{(75:25)}$  and  $RAM_{(50:50)}$  samples. It was determined that as the almond milk rate of samples increased, the consistency values of samples value decreased. Since cohesiveness is related to the strength of the internal bonds in yogurt structure, the lower cohesiveness the smoother yogurt texture. Increasing almond milk rate of samples resulted in a decrease of the cohesiveness values of samples. The cohesiveness values of  $RM_{(100)}$  and  $RAM_{(75:25)}$  samples during storage showed statistically insignificant differences ( $P > 0.05$ ), while those of  $RAM_{(25:75)}$  and  $AM_{(100)}$  samples decreased after 7th-day of storage ( $P < 0.05$ ). Regarding with cohesiveness, similar results were reported by Arslan (2018) who found that peanut milk resulted in decreasing the cohesiveness in yogurt. The higher values of index of viscosity refer to being more resistant to gradual deformation of the sample by shear stress, meaning thickness. There were significant differences in yogurt type and storage time for viscosity values ( $P < 0.05$ ). During storage, the viscosity values of  $RM_{(100)}$  and  $RAM_{(25:75)}$  samples decreased, while those of  $RAM_{(75:25)}$  and  $RAM_{(50:50)}$  samples increased. A greater values in the the index of viscosity were observed until day 7 of storage for  $RM_{(100)}$  sample than other samples and this value for  $RM_{(100)}$  decreased thereafter until day 21. Textural properties of yogurt are influenced mainly by solid content, physical states of fats and proteins in milk, milk composition, temperature and time of heating, homogenization, mechanical handling of coagulum, use of stabilizers, type and quantity of starter culture, acidity, degree of proteolysis, fermentation and storage conditions (Lee and Lucey 2010; Ozcan 2013; Yilmaz-Ersan et al. 2017). In this study, the textural differences were found to be significant among samples, depending on the almond milk rate it was observed that textural parameters decreased, which results in an decrease in the curd strength of yogurt. The results of texture analysis obtained during storage suggested that supplementation of almond milk resulted in an unstable system and the formation of a weak three-dimensional network in yogurt. Thus, further studies are required to characterize hydrophilic properties, the protein network, physical states of fats and proteins, protein-polysaccharide interactions, and gelation behavior in

**Table 2** The instrumental texture parameters of the probiotic yoghurts during storage

Texture parameters	Treatments	Storage period (day)			
		1	7	14	21
Firmness (g)	RM <sub>(100)</sub>	267.70 <sup>aA</sup>	339.21 <sup>aA</sup>	361.20 <sup>aA</sup>	377.85 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	197.80 <sup>bA</sup>	280.64 <sup>aA</sup>	276.42 <sup>abA</sup>	324.75 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	121.83 <sup>cB</sup>	104.14 <sup>bB</sup>	189.45 <sup>bA</sup>	118.22 <sup>bB</sup>
	RAM <sub>(25:75)</sub>	37.02 <sup>dA</sup>	32.29 <sup>bcB</sup>	32.65 <sup>cB</sup>	32.26 <sup>bB</sup>
	AM <sub>(100)</sub>	11.84 <sup>dA</sup>	11.33 <sup>cA</sup>	11.71 <sup>cA</sup>	11.21 <sup>bA</sup>
Consistency (g·sec)	RM <sub>(100)</sub>	5931.78 <sup>aC</sup>	6578.71 <sup>aB</sup>	7123.61 <sup>aA</sup>	7030.75 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	3882.09 <sup>bB</sup>	4514.01 <sup>bA</sup>	4840.77 <sup>bA</sup>	5031.53 <sup>bA</sup>
	RAM <sub>(50:50)</sub>	1988.34 <sup>cC</sup>	2176.40 <sup>cB</sup>	2354.14 <sup>cAB</sup>	2505.86 <sup>cA</sup>
	RAM <sub>(25:75)</sub>	244.56 <sup>dB</sup>	264.55 <sup>dAB</sup>	274.03 <sup>dA</sup>	267.24 <sup>dAB</sup>
	AM <sub>(100)</sub>	68.69 <sup>dA</sup>	67.96 <sup>dA</sup>	68.27 <sup>dA</sup>	68.55 <sup>dA</sup>
Cohesiveness (g)	RM <sub>(100)</sub>	- 103.63 <sup>aA</sup>	- 99.86 <sup>aA</sup>	- 99.03 <sup>aA</sup>	- 96.56 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	- 99.75 <sup>aA</sup>	- 96.73 <sup>aA</sup>	- 102.31 <sup>aA</sup>	- 94.79 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	- 98.92 <sup>aA</sup>	- 71.13 <sup>bB</sup>	- 101.15 <sup>aA</sup>	- 73.44 <sup>bB</sup>
	RAM <sub>(25:75)</sub>	- 23.43 <sup>bA</sup>	- 21.88 <sup>cA</sup>	- 17.78 <sup>bB</sup>	- 17.12 <sup>cB</sup>
	AM <sub>(100)</sub>	- 6.81 <sup>bAB</sup>	- 7.09 <sup>dA</sup>	- 6.27 <sup>bB</sup>	- 6.65 <sup>dAB</sup>
Index of viscosity (g·sec)	RM <sub>(100)</sub>	- 469.12 <sup>aA</sup>	- 459.35 <sup>aB</sup>	- 1.26 <sup>cC</sup>	- 0.79 <sup>cC</sup>
	RAM <sub>(75:25)</sub>	- 297.87 <sup>bB</sup>	- 329.40 <sup>bAB</sup>	- 364.06 <sup>aA</sup>	- 359.07 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	- 117.08 <sup>cC</sup>	- 116.67 <sup>cC</sup>	- 167.53 <sup>bA</sup>	- 142.50 <sup>bB</sup>
	RAM <sub>(25:75)</sub>	- 9.56 <sup>dA</sup>	- 8.21 <sup>dA</sup>	- 6.57 <sup>cB</sup>	- 6.32 <sup>cB</sup>
	AM <sub>(100)</sub>	5.81 <sup>dA</sup>	5.72 <sup>cA</sup>	6.153 <sup>cA</sup>	5.80 <sup>cA</sup>

<sup>a-d</sup>Different lowercase supercripts in the same column depict the significant difference between means for samples ( $P < 0.05$ )

<sup>A-D</sup>Different uppercase supercripts in the same row depict the significant difference between means for storage time ( $P < 0.05$ )

almond milk which may be responsible for the elevated textural parameters.

Yogurt, a ready fresh food, doesn't need any treatments such as washing, cooking and freezing, thus, the sensory quality is one of the determinants of a consumer's choice after manufacturing and during storage. Consumers demand yogurt with a refreshing flavor, a smooth viscous gel, and a slight sour taste. In order to assess the acceptability of the probiotic yogurt with almond milk, sensory evaluation was carried out. The data of colour, appearance, structure, texture, odour, taste and flavor, sensorial acidity and overall acceptability were presented in Table 3. The statistical differences in samples were significant ( $P < 0.05$ ). The panellists' ratings for all of the sensorial parameters tended to decrease with increasing almond milk levels. Compared with storage days, generally there were no statistically significant differences ( $P > 0.05$ ) in panellist ratings except for appearance and taste/flavor in RAM<sub>(25:75)</sub> sample, odor and overall acceptability in RAM<sub>(50:50)</sub> sample. According to the panellist descriptions, almond milk substitution resulted in the occurrence of nutty, sweetness and a little bitterness. Syneresis, higher

water separation, was observed on the appearance of RAM<sub>(25:75)</sub> and AM<sub>(100)</sub> samples, whereas this defective factor had a lesser effect on probiotic yogurts including reconstituted milk. Regarding the texture of yogurt, sensory evaluation was performed on structure and textural characteristics in order to obtain data that could support the texture profile analysis. Similar to appearance, the increasing of almond milk ratios decreased the sensorial textural parameters. At the end of storage period, RM<sub>(100)</sub> sample was still better liked by the consumers and thus being generally acceptable to the panellists it gained the highest score. Similar results were mentioned by Ilyasoglu and Yilmaz (2019) that the overall acceptability scores of yogurt enriched with hazelnut milk were lower than those of a control yogurt from skim milk. Fatima and Hekmat (2020) investigated sensorial properties of yogurt mixtures prepared by incorporating 0%, 25%, 50%, or 75% soymilk in cow milk. They mentioned that yogurts including a higher percentage of cow milk were found to be higher hedonic scores for appearance and texture. As a conclusion, the addition of 25% of almond milk to yogurt was most appreciated by the panellists. However some

**Table 3** The sensorial evaluation of the probiotic yoghurts during storage

Sensorial parameters	Treatments	Storage period (day)			
		1	7	14	21
Colour	RM <sub>(100)</sub>	5.00 <sup>aA</sup>	4.94 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.90 <sup>aA</sup>	4.96 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.76 <sup>abA</sup>	4.70 <sup>aA</sup>	4.98 <sup>aA</sup>	4.76 <sup>abA</sup>
	AM <sub>(100)</sub>	4.32 <sup>bA</sup>	3.54 <sup>bA</sup>	4.14 <sup>bA</sup>	4.32 <sup>bA</sup>
Appearance	RM <sub>(100)</sub>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	4.96 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.96 <sup>aA</sup>	4.76 <sup>aA</sup>	5.00 <sup>aA</sup>	4.98 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.10 <sup>abB</sup>	4.22 <sup>aAB</sup>	4.90 <sup>aA</sup>	4.10 <sup>bB</sup>
	AM <sub>(100)</sub>	3.40 <sup>bA</sup>	2.80 <sup>bA</sup>	3.38 <sup>bA</sup>	2.90 <sup>cA</sup>
Structure/body	RM <sub>(100)</sub>	5.00 <sup>aA</sup>	4.76 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	5.00 <sup>aA</sup>	4.90 <sup>aB</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.82 <sup>abA</sup>	4.74 <sup>aA</sup>	4.86 <sup>abA</sup>	4.78 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.24 <sup>bA</sup>	4.04 <sup>aA</sup>	4.20 <sup>bA</sup>	3.66 <sup>bA</sup>
	AM <sub>(100)</sub>	3.00 <sup>cA</sup>	2.40 <sup>bA</sup>	3.26 <sup>cA</sup>	2.46 <sup>cA</sup>
Texture	RM <sub>(100)</sub>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	5.00 <sup>aA</sup>	4.86 <sup>aA</sup>	5.00 <sup>aA</sup>	4.92 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.80 <sup>abA</sup>	4.66 <sup>aA</sup>	4.86 <sup>aA</sup>	4.70 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.24 <sup>bA</sup>	4.06 <sup>aA</sup>	4.40 <sup>aA</sup>	3.82 <sup>bA</sup>
	AM <sub>(100)</sub>	3.00 <sup>cA</sup>	2.20 <sup>bA</sup>	3.26 <sup>bA</sup>	2.68 <sup>cA</sup>
Odour	RM <sub>(100)</sub>	4.90 <sup>aA</sup>	5.00 <sup>aA</sup>	4.98 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	4.96 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>	5.00 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.76 <sup>abB</sup>	4.96 <sup>aAB</sup>	5.00 <sup>aA</sup>	4.84 <sup>abAB</sup>
	RAM <sub>(25:75)</sub>	4.72 <sup>abA</sup>	4.58 <sup>abA</sup>	4.96 <sup>aA</sup>	4.86 <sup>abA</sup>
	AM <sub>(100)</sub>	4.28 <sup>bA</sup>	4.08 <sup>bA</sup>	4.64 <sup>aA</sup>	4.36 <sup>bA</sup>
Taste and Flavor	RM <sub>(100)</sub>	4.96 <sup>aA</sup>	4.78 <sup>aA</sup>	4.88 <sup>aA</sup>	4.76 <sup>abA</sup>
	RAM <sub>(75:25)</sub>	4.98 <sup>aA</sup>	4.80 <sup>aA</sup>	4.94 <sup>aA</sup>	4.94 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.84 <sup>aA</sup>	4.70 <sup>aA</sup>	4.94 <sup>aA</sup>	4.82 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.16 <sup>abA</sup>	4.32 <sup>abA</sup>	4.30 <sup>abA</sup>	4.34 <sup>bcA</sup>
	AM <sub>(100)</sub>	3.52 <sup>bA</sup>	3.10 <sup>bA</sup>	3.56 <sup>bA</sup>	4.00 <sup>cA</sup>
Sourness	RM <sub>(100)</sub>	4.94 <sup>aA</sup>	4.94 <sup>aA</sup>	4.78 <sup>aA</sup>	4.78 <sup>abA</sup>
	RAM <sub>(75:25)</sub>	4.96 <sup>aA</sup>	4.96 <sup>aA</sup>	4.90 <sup>aA</sup>	4.80 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.72 <sup>aA</sup>	4.78 <sup>aA</sup>	4.90 <sup>aA</sup>	4.92 <sup>aA</sup>
	RAM <sub>(25:75)</sub>	4.32 <sup>aA</sup>	4.34 <sup>aA</sup>	4.60 <sup>aA</sup>	4.34 <sup>bcA</sup>
	AM <sub>(100)</sub>	3.56 <sup>bA</sup>	3.40 <sup>bA</sup>	4.18 <sup>aA</sup>	3.92 <sup>cA</sup>
Overall acceptability	RM <sub>(100)</sub>	4.96 <sup>aA</sup>	4.94 <sup>aA</sup>	4.96 <sup>aA</sup>	4.96 <sup>aA</sup>
	RAM <sub>(75:25)</sub>	4.98 <sup>aA</sup>	4.92 <sup>aA</sup>	4.98 <sup>aA</sup>	4.94 <sup>aA</sup>
	RAM <sub>(50:50)</sub>	4.84 <sup>aAB</sup>	4.64 <sup>aB</sup>	4.98 <sup>aA</sup>	4.88 <sup>aAB</sup>
	RAM <sub>(25:75)</sub>	4.24 <sup>aA</sup>	3.86 <sup>aA</sup>	4.96 <sup>aA</sup>	4.26 <sup>aA</sup>
	AM <sub>(100)</sub>	3.16 <sup>bA</sup>	2.50 <sup>bA</sup>	3.36 <sup>bA</sup>	3.00 <sup>bA</sup>

<sup>a–d</sup>Different lowercase supercripts in the same column depict the significant difference between means for samples ( $P < 0.05$ )

<sup>A–D</sup>Different uppercase supercripts in the same row depict the significant difference between means for storage time ( $P < 0.05$ )

**Table 4** Pearson's correlation coefficients among all the variables

	L*	a*	b*	H	C	ΔE*	F	CS	CHV	IV	Colour	Appearance	Structure	Texture	Odor	T/F	Sourness	OA
L*	1																	
a*	-0.975**	1																
b*	0.437	-0.622	1															
H	0.976**	-0.907*	0.236	1														
C	0.572	-0.737	0.988**	0.385	1													
ΔE*	-0.998**	0.989**	-0.229	-0.973*	-0.438	1												
F	-0.813	0.915*	0.873**	0.666	0.937**	-0.805	1											
CS	-0.783	-0.896*	0.893*	0.631	0.950**	-0.788	0.813	1										
CHV	-0.855	0.927*	-0.738	-0.761	-0.821	0.847	-0.909*	-0.883*	1									
IV	-0.773	0.875	-0.757	-0.648	-0.822	0.770	-0.910*	-0.865*	0.916*	1								
Colour	0.978**	-0.925*	0.294	0.993**	0.438	-0.986**	0.698	0.660	-0.821	-0.700	1							
Appearance	0.978**	-0.955**	0.415	0.967**	0.548	-0.986**	0.772	0.736	-0.896*	-0.773	0.989**	1						
Structure	0.976**	-0.967**	0.474	0.950**	0.602	-0.984**	0.809	0.774	-0.924*	-0.814	0.977**	0.997**	1					
Texture	0.987**	-0.973**	0.460	0.963**	0.591	-0.991**	0.809	0.776	-0.907*	-0.796	0.983**	0.998**	0.998**	1				
Odor	0.993**	-0.961**	0.399	0.980**	0.536	-0.999**	0.780	0.742	-0.868*	-0.782	0.991**	0.992**	0.989**	0.994**	1			
T/F	0.963**	-0.957**	0.477	0.937**	0.602	-0.945*	0.802	0.764	-0.933*	-0.822	0.970**	0.995**	0.999**	0.993**	0.981**	1		
Sourness	0.968**	-0.959**	0.468	0.944**	0.595	-0.979*	0.800	0.763	-0.927*	-0.815	0.975**	0.997**	0.999**	0.995**	0.985**	1.000**	1	
OA	0.980**	-0.964**	0.445	0.961**	0.576	-0.987**	0.793	0.758	-0.900*	-0.792	0.984**	0.999**	0.999**	0.992**	0.996**	0.998**	0.998**	1

F Firmness, CS Consistency, CHV Cohesiveness, IV Index of viscosity, T/F Taste and flavor, OA Overall acceptability \*Correlation is significant at the 0.05 level, \*\* Correlation is significant at the 0.01 level

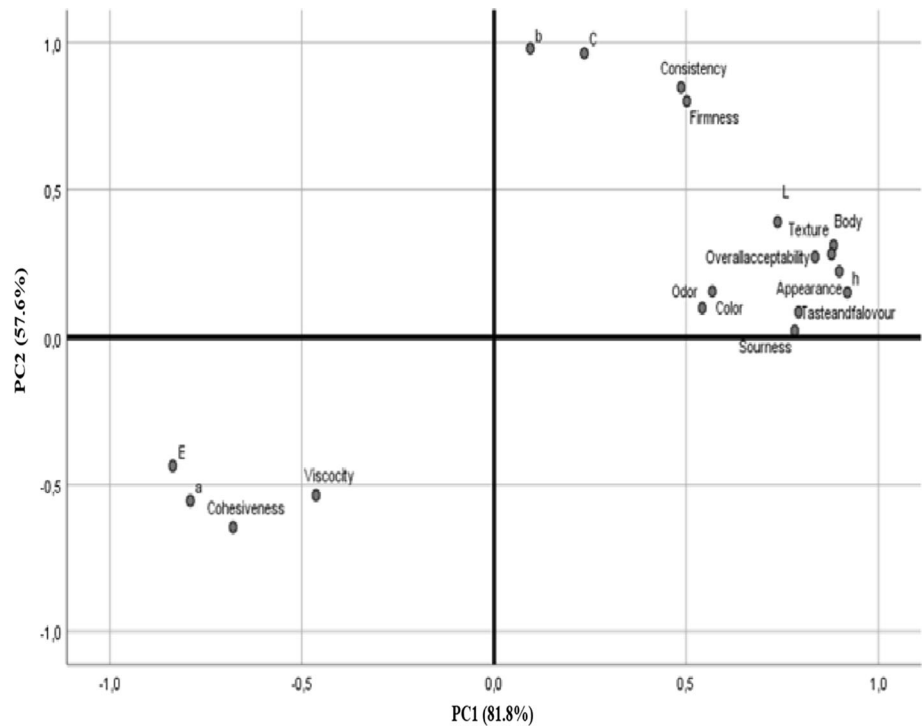
modifications (addition of stabilizers, sweeteners, flavorings and fruits) in the yogurt formula could help to improve all sensorial aspects and marketability of probiotic yogurt enriched with almond milk.

Pearson's correlation coefficients were used to understand the use of almond milk in yogurt between instrumental measurements and sensory attributes which were presented in Tables 1, 2 and 3. L\* was significantly positively correlated with H\* ( $r = 976, P < 0.01$ ) and all of sensorial parameters ( $P < 0.01$ ). a\* was significantly positively correlated with ΔE\* ( $r = 989, P < 0.01$ ), while negatively correlated with all of sensorial parameters ( $P < 0.01$ ). b\* was also significantly correlated with C\* ( $r = 0.988, P < 0.01$ ), firmness ( $r = 0.873, P < 0.05$ ) and consistency ( $r = 0.893, P < 0.05$ ). A significant positive correlation between H\* and sensorial parameters were observed (Table 4.). C\* was significantly positively correlated with firmness ( $r = 937, P < 0.01$ ) and consistency ( $r = 0.950, P < 0.01$ ) ΔE\* and all of sensorial parameters were significantly negatively correlated ( $P < 0.01, P < 0.05$ ). Significant negative correlation was observed between firmness and cohesiveness ( $r = -0.909, P < 0.05$ ) as well as index of viscosity ( $r = -0.910, P < 0.05$ ). Cohesiveness and almost all sensorial parameters were negatively correlated ( $P < 0.05$ ). Significant positive correlation among the sensorial parameters was also observed ( $P < 0.01$ ). Generally, the parameters of firmness, consistency and index of viscosity did not significantly correlate with sensory properties ( $P > 0.05$ ). The non-linear correlations of these parameters can be attributed to differences between the oral perception process and instrumental methodology used.

Principal component analysis (PCA) of attributes analysed in this study helped to visualize the differences among probiotic yogurt samples (Fig. 1). PC1 accounted for 81.8% of the variation, and seemed to differentiate among samples according to sourness, taste and flavor, color, odor, appearance, H, overall acceptability, texture, structure/body, L\*, firmness, consistency, b\* and C attributes. Consequently, the sensory characteristics of all the samples are mainly explained by the positive side of principal component 1 (PC1). PC2 accounted for 57.6% of the variation, and seemed to differentiate among samples according to ΔE\*, a\*, cohesiveness and index of viscosity. The use of almond milk in probiotic yogurt production showed negative coordinates characterized by attributes ΔE\*, a\*, cohesiveness and index of viscosity of the samples. Therefore, the results of this study indicate the potential of using almond milk combined with animal milk to achieve the desired sensory properties of product. However, further optimizing is required to obtain yogurts with satisfactory textural properties and an appealing appearance that would satisfy consumer preference.



**Fig. 1** Principal component analysis representing attributes analysed for the probiotic yoghurt samples



**Fig. 2** Classification of probiotic yoghurt samples by HCA on the evaluated variables

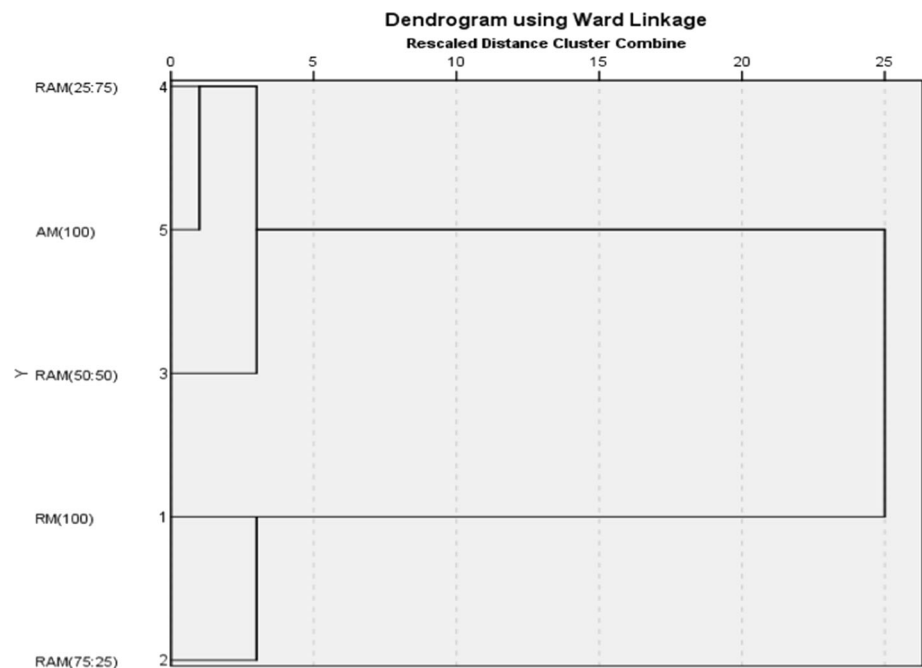


Figure 2 showed the dendrogram obtained from Hierarchical Cluster Analysis performed to classify the analysed attributes for probiotic yogurt samples. Five samples were grouped into two big clusters based on the mean values generated from the unweighted pair group mean average method of analysis. The results obtained showed that

RM<sub>(100)</sub> and RAM<sub>(75:25)</sub> samples were in the same cluster. This cluster included yogurt including 100% reconstituted milk and yogurt with 75% reconstituted milk plus 25% almond milk which were more similar according to all of attributes analysed in this study. The other cluster is composed of RAM<sub>(25:75)</sub>, AM<sub>(100)</sub> and RAM<sub>(50:50)</sub> samples.

Thus, it was considered that the use of almond milk in probiotic yogurt production resulted in important effects on probiotic yoghurt consumption.

## Conclusion

The supplementation of almond milk into dairy products produces functional food and meets the expectations of consumers demanding food products with high nutritional value. Almond milk proved its efficient use as an innovative and attractive additive in probiotic yogurt processing. The use of different concentrations of almond milk in probiotic yogurt production affected the instrumental measurements (color and textural parameters) and the sensory attributes of the product. From hedonic analysis results, the panellists appreciated that almond milk could be incorporated into yogurt to a level of 25%. Correlations were observed between instrumental characterization and sensorial evaluation. The grouping of variables in PCA plots indicated specific yogurts properties, which are important for their potential commercial or industrial use. Further research should be carried out to improve the textural and sensorial properties of yogurt with almond milk.

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## Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflict of interest.

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