



Physicochemical and sensory evaluation of greek style yoghurt with bovine colostrum

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Abstract

This study aimed to develop Greek style yoghurts with the addition of bovine colostrum. Four yoghurt formulations were produced with different colostrum content (0%, 10%, 20% and 30%). The fat, protein, total solids, ash, retinol levels were quantified as well as the colour was measured. A sensory analysis was conducted with 103 untrained panelists, evaluating the attributes of appearance, aroma, consistency, flavour, overall acceptance and the purchase intention. Colostrum addition increased the fat and protein levels in the yoghurt formulations. The Greek style yoghurts with bovine colostrum reached sensory acceptance above 70% for all attributes.

Keywords: bovine colostrum; sensory acceptance; fermented products; dairy products; chemical composition.

Practical Application: Use of colostrum as a source of bioactive compounds for adding value to food products, especially dairy such as Greek style yoghurt.

1 Introduction

Colostrum is the first secretion of the mammary gland from 2 to 4 days after calving and differs from milk because of the greater amounts of fat, protein, minerals and vitamins, in addition to a high concentration of immunoglobulins that provide passive immunity to the newborn. This specific composition of colostrum may reveal particular biological activities. Antioxidant activity is among the fundamental biological activities important for life (Oussaief et al., 2020).

The use of bovine colostrum in food and supplements for human consumption has attracted the attention of the scientific community due to its properties in fighting and preventing infections and diseases. Colostrum is a food rich in immunoglobulins, mainly IgG, and has great potential to be used in producing human foods (Sahana et al., 2018; Ceniti et al., 2019). Thus, food products with the addition of colostrum have the potential to arouse the interest of the food industry and the consumer, whose eating habits have been changing over the last few decades. There is currently a greater demand for foods with functional properties which are rich in compounds that produce health benefits (Conte & Scarantino, 2013).

In addition to a higher concentration of proteins and immunoglobulins, dairy derivatives with the addition of bovine colostrum have a lower amount of lactose when compared to

foods produced with mature milk (Ahmadi et al., 2011). Some products based on bovine colostrum have been marketed for many years as food supplements in countries such as New Zealand, the United States, Europe and China (Cao et al., 2007).

Yoghurt is a product whose fermentation takes place with protosymbiotic cultivations of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* to which other acid-lactic bacteria can be added in a complementary way, determining the characteristics of the final product (Brasil, 2000). In addition, the use of fermented foods, such as yoghurt provides a number of positive effects on human health due to lactic acid bacteria (LAB) and have been considered as important food supplements for bone health due to the high content of bioavailable calcium (Lee et al., 2020). Lactic acid bacteria in the presence of prebiotics were found to be effective against increased blood lipid profile such as total cholesterol, triglycerides, LDLs and HDLs (Sarraz et al., 2019).

In turn, Greek style yoghurt is characterized by having a higher percentage of solids due to the removal of acid whey by leaching the yoghurt curd (Bong & Moraru, 2014). Producing yoghurt enriched with bovine colostrum is an interesting alternative, since it raises the nutritional values of yoghurt and provides immunological benefits to the consumer (Abdel-Ghany & Zaki, 2018).

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In view of the nutritional and functional importance of colostrum, the objective of the present study was to evaluate the chemical composition, sensory acceptance and purchase intention of different Greek style yoghurt formulations with the addition of bovine colostrum.

2 Materials and methods

2.1 Materials and experimental design

In this pilot study, four different Greek style yoghurt (GY) formulations were prepared with different concentrations of bovine colostrum collected in the third milking (24 hours after the calving). The formulations were named C0 (100:0 v:v, milk:colostrum – control), C10 (90:10 v:v, milk:colostrum), C20 (80:20 v:v, milk:colostrum) and C30 (70:30 v:v, milk:colostrum). Jersey bovine milk and colostrum were purchased from a commercial property in the municipality of São Gonçalo do Amarante, Rio Grande do Norte, Brazil.

The GY formulations were developed at the Milk and Derivatives Processing Unit, which belongs to the Universidade Federal do Rio Grande do Norte, Campus Macaíba, RN, Brazil. The GY production stages followed the norms of Normative Instruction no. 46 (Brasil, 2007) and were carried out as described by Pereda (2005), with some adaptations. The production flowchart is shown in Figure 1.

Briefly, 10 g/L soluble solids (powdered milk) and 8 g/L sucrose were added to each formulation (C0, C10, C20 and C30). After homogenization, the mixture was subjected to heat treatment (60 °C for 45 min) in a water bath. After cooling to 43 ± 1 °C in an

ice bath, the culture (10 ml/L) was inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* microorganisms. Then, the fermentation step (43 ± 1 °C for 18 h) was carried out in a B.O.D incubator (TE - 390, Tecnal, Brazil) until reaching a pH of 4.6. Soon after, the samples were cooled in a cold chamber at 5 °C for 30 minutes and poured into glass flasks coupled with a filter for draining for 24 hours. At the end of GY production, the samples were stored in 2-liter plastic pots identified according to the colostrum concentration and kept in a cold chamber at 5 °C.

2.2 Chemical composition analysis

Protein analysis was performed according to the Kjeldahl method (Association of Official Analytical Chemists, 2012), using a factor of 6.25 for conversion to protein nitrogen. The fat content was determined by the Gerber method (Brasil, 2006). The total solids and ash were analyzed by gravimetric method (Association of Official Analytical Chemists, 2012). All analyzes were performed in triplicate.

2.3 Retinol quantification by high performance liquid chromatography (HPLC)

An average of 1 g of GY was weighed in polypropylene tubes protected from light for the yoghurt extraction process. Next, vitamins were extracted according to Giuliano et al. (1992), with modifications. Briefly, 1 mL of 95% ethanol and 1 mL of 50% KOH were added for protein denaturation and hydrolysis of retinol esters, respectively. Then, the tubes were shaken for 1 minute and left in a 60 °C water bath for 60 minutes, being shaken every 15 minutes.

Next, 2 mL of hexane were added as an extractive reagent and stirred for 1 minute. The tubes were subsequently centrifuged for 10 minutes, the supernatant hexane phase was removed and transferred to another 15 mL tube. This procedure was repeated twice, making a total of 6 mL of the hexane extract. After this step, the hexane extract was stirred and a 4 mL aliquot was removed, which was evaporated in a water bath at 37 °C under a nitrogen atmosphere. The dry extract was redissolved in 250 µL of absolute ethanol.

The retinol concentration was determined by High Performance Liquid Chromatography (HPLC) in a LC-10 AD Shimadzu Chromatograph, coupled to a Shimadzu UV-VIS SPD-10A Detector and Shimadzu C-R6A Integrator with a C18 LC Shim-pack CLC -ODS (M) 4.6 mm × 25 cm. The mobile phase used was 100% methanol with a flow rate of 1 mL/min. The retinol in the samples was identified and quantified by comparison with the retention times and areas of the respective standards. The standard concentration was confirmed by the specific extinction coefficient (ϵ 1%, 1cm = 1780) in absolute ethanol and a wavelength of 325 nm (Nierenberg & Nann, 1992).

2.4 Colour measurement

The colour of GY samples was measured by reflectance spectrophotometry using an ACR-1023 colorimeter (Instrutherm, São Paulo, Brazil) in the RGB system and converted to CIELab by

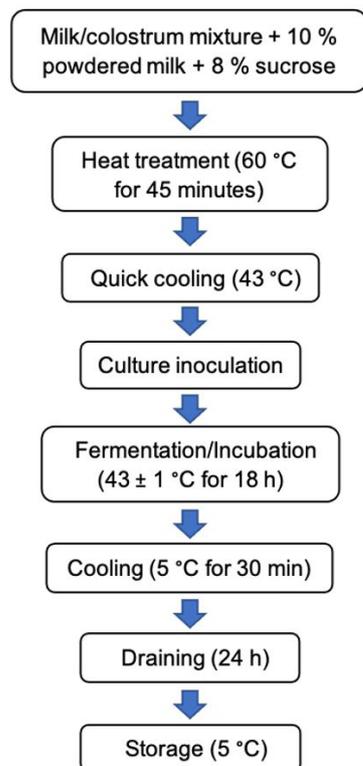


Figure 1. Greek style yoghurt production flowchart.

the OpenRGB[®] program. Following this method, the L*, a* and b* coordinates were recorded.

2.5 Sensory analysis

A nine-point hedonic scale test (1- dislike extremely; 2- dislike very much; 3- dislike moderately; 4- dislike slightly; 5- neither like nor dislike; 6- like slightly; 7- like moderately; 8- like very much; 9- like extremely) was used to evaluate sample acceptability. A sensory assessment of GY samples was done after five days of cold storage. The panelists (n = 103) were members of the staff and students of the Universidade Federal do Rio Grande do Norte (UFRN), Natal, Brazil. The samples (50 mL) were presented monadically following a completely randomized design in disposable cups. The samples were provided at 10 °C. Each panelist was instructed to evaluate appearance, flavour, texture, taste and overall liking. Mineral water and cream cracker biscuits were available as neutralizers between samples in order to avoid carryover effects. The panelists were asked to express their purchase intention towards each sample, marking “yes”, “maybe” or “no” for the question “Would you buy this product if it was available on the market?” Tests were conducted in the Laboratory of Sensory Analysis, Department of Nutrition, UFRN.

The Acceptability Index (AI) was calculated as suggested by Dutcosky (2007), who classifies a product with good acceptability when the mean values of sensory properties are greater than 70%. AI was calculated according to Equation 1:

$$AI (\%) = M / h \times 100 \quad (1)$$

In which: AI = Acceptability Index; M = arithmetic mean of the scores assigned to the assessed characteristic; h = highest score attributed by the panelists to the assessed characteristic.

2.6 Ethical aspects

This study was approved by the Animal Use Ethics Committee of the Universidade Federal do Rio Grande do Norte, under protocol no. 098.023/2018. The protocol was also approved by the Research Ethics Committee Involving Humans at the Federal University of Rio Grande do Norte under the protocol no. 94022818.3.0000.5537.

2.7 Statistical analysis

The composition and sensory evaluation data were submitted to one-way analysis of variance (ANOVA), complemented by the Duncan test ($P < 0.05$), using the SAS version 9.0 software program. Purchase intention was assessed using descriptive statistics.

3 Results and discussion

3.1 Chemical composition

The protein, fat, total solids and ash contents among the GY formulations are shown in Table 1. Among the formulations, no statistical differences were observed between the averages for protein, total solids and ash. The GY without added colostrum

(C0) had a lower fat percentage ($P < 0.05$) when compared to the other formulations (Figure 2).

There was a statistical difference in the protein percentage ($P < 0.05$) between the sample with the highest colostrum addition and the other evaluated formulations. In this sense, the C0 sample had the lowest percentage (14.96 g/100g), while C30 exhibited an exponential increase (19.61 g/100 g) in comparison to the yoghurts with lower quantity of colostrum addition. Such findings corroborate the study by Ayar et al. (2016) in yoghurt and kefir made with different colostrum levels in which the authors observed a gradual increase in protein content, although the differences were not statistically significant. In a study conducted by Abdel-Ghany & Zaki (2018), high level of yoghurt protein was associate with the increase of colostrum percentage in yoghurt. Thus, enriching milk by adding colostrum is a potential strategy to produce dairy products with a higher protein concentration.

The averages for fat content ranged from 8.83 g/100 g (C0) to 11.50 g/100 g (C10). The samples enriched with colostrum showed higher values ($P < 0.05$) than that without the colostrum

Table 1. Chemical composition of Greek style yoghurt with the addition of bovine colostrum (mean \pm SD).

	C0	C10	C20	C30
Protein (g/100g)	14.96 \pm 0.72 ^b	15.32 \pm 2.74 ^{ab}	15.59 \pm 0.74 ^{ab}	19.61 \pm 1.28 ^a
Fat (g/100g)	8.33 \pm 1.04 ^c	11.50 \pm 0.50 ^a	10.17 \pm 0.29 ^b	10.17 \pm 0.29 ^b
Total solids (g/100g)	31.08 \pm 0.97 ^a	31.60 \pm 0.37 ^a	31.43 \pm 0.99 ^a	31.28 \pm 0.47 ^a
Ash (g/100g)	4.46 \pm 0.16 ^a	3.59 \pm 1.10 ^a	4.53 \pm 0.52 ^a	4.30 \pm 0.16 ^a
Retinol (μ g/100g)	69.93 ^a	62.60 ^a	68.87 ^a	68.20 ^a

Means and standard deviation with different letters on each specific component differ by the Duncan test ($P < 0.05$). C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

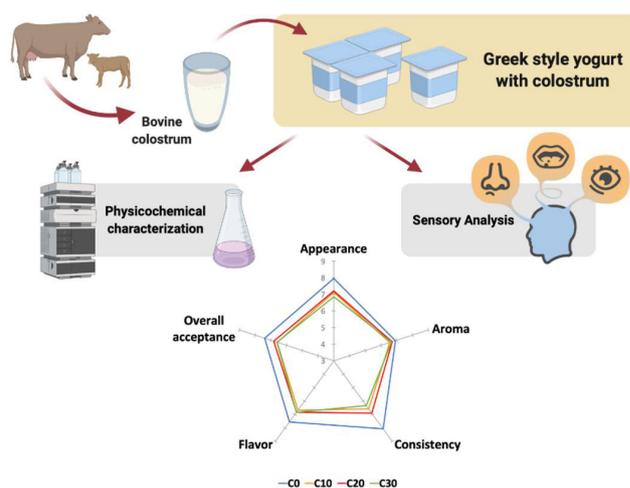


Figure 2. Development and evaluation of Greek style yoghurts with bovine colostrum. C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

addition, meaning that colostrum contributes to lipid enrichment. Similar results have been reported by Ayar et al. (2016) for yoghurt and kefir samples with added bovine colostrum, and by Abdel-Ghany & Zaki (2018) in fortified yoghurt with the addition of different bovine colostrum concentrations. The fat concentration of GYs developed in this study exceeds Greek style yoghurts of different commercial brands (3.9 to 6.3 g /100 g) (Magalhães & Della Torre, 2018). However, we did not observe any linear increase in the fat content of GY increasing colostrum levels in the formulations. In the study of Setyawardani et al. (2020), no significant differences were observed in fat content among kefir samples produced with increasing bovine colostrum percentages, which were even higher than those used in our study (0, 20, 40, 60, 80 and 100% colostrum).

There were no significant differences for total solids among GY formulations, where the C0 presented the lowest value of total solids (31.08 g/100 g), while the C10 had the highest amount (31.60 g/100 g). We obtained higher level of total solids than that reported by Magalhães & Della Torre (2018), with a range from 15.53 to 28.20 g/100 g, and Desai et al. (2013) with total solids content in commercial Greek style yoghurts below than 23.8%. The ash concentration ranged from 3.59 g/100 g (C10) to 4.53 g/100 g (C20), which is higher than Greek style yoghurt with the addition of Pequi (*Caryocar brasiliense*) (1.15 to 1.24 g/100 g) in a work developed by Silva et al. (2014). Regarding the total solids and ash content being higher than values reported in the literature, the presence of colostrum did not contribute to any clear increase in these components in GY formulations, even though it contributed to higher protein and fat concentrations.

There were no statistical differences for the retinol concentration among GY formulations. However, the levels observed in the present study are higher than the minimum reported by Philippi (2002) for natural yoghurt. In addition, our results are close to the values reported by Ribeiro et al. (2007), who evaluated the retinol levels in different natural yoghurt brands sold in the city of Natal-RN (47.6 and 65.1 µg/100 mL, respectively).

Table 2 shows the colour measurements for GYs with different bovine colostrum additions. The samples are similar in relation to the a^* parameter, with values close to zero, which means low intensity of green and red colours.

The C30 sample presented higher means of L^* and b^* ($P < 0.05$) when compared to GY formulations with lower colostrum, while C0, C10 and C20 were similar to each other. Indeed, GY sample with the highest colostrum percentage

Table 2. Color analysis of Greek style yoghurt with different bovine colostrum additions.

Formulation	L^*	a^*	b^*
C0	32.22 ^b	-3.49 ^a	9.62 ^b
C10	32.09 ^b	-2.83 ^a	10.07 ^b
C20	30.84 ^b	-2.55 ^a	9.14 ^b
C30	42.15 ^a	-3.80 ^a	14.51 ^a

Means and standard deviation with different letters on the same line differ by the Duncan test ($P < 0.05$). C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

(C30) resulted the lightest. Regarding b^* , all samples showed a yellowish colour, especially C30 samples ($P < 0.05$).

Such results corroborate the findings of Ayar et al. (2016), in which the authors observed that the samples with the highest addition of colostrum for yoghurt and kefir were those that reached the highest values for L^* and b^* and lowest for a^* . This behavior reflects the typical yellowish colour of colostrum which affects the resulting food (Ayar et al., 2016). In contrast, in a study conducted with yoghurt produced with different colostrum additions (0%, 5%, 10% and 15%), Abdel-Ghany & Zaki (2018) observed higher luminosity values (L^*) for the control treatment samples without colostrum addition; however, the authors observed higher b^* values in samples with colostrum additions in 10 and 15%, which can be compared to the present study.

3.2 Sensory analysis

Table 3 presents the scores attributed by the sensory panel of Greek style yoghurts produced with different bovine colostrum concentrations (Figure 2). Yoghurt without added colostrum (C0) obtained the highest values for overall acceptance of 7.39, different from samples with bovine colostrum addition. We observed averages for overall acceptance of 6.29 for the sample with 10% bovine colostrum addition, 6.81 for the sample with 20% bovine colostrum addition, and 6.60 for the sample with 30% bovine colostrum addition, being evaluated with scores anchored in the nominal scale of “I slightly liked it”.

The C0 GY had higher scores for the appearance (7.96), consistency (8.06) and flavour (7.56) attributes, followed by the C20 which presented averages of 7.20 for appearance, 6.90 for consistency and 6.83 for flavour attributes, respectively. The C30 sample had the lowest sensory scores for both the appearance (6.85) and Consistency (6.34) attributes.

The food colour is one of the factors which influences the product appearance, and consumers prefer to have traditional Greek style yoghurts with a lighter colour (Magalhães & Della Torre, 2018), so that the colostrum addition in the yoghurt samples may have contributed to reducing the mean sensory scores referring to appearance, depending on the yellowish coloration typical of colostrum (Ayar et al., 2016). This behavior was observed by evaluating the colour of Greek style yoghurts, in which the C30 sample had the highest values for b^* , meaning that it presented the most yellowish colour among the evaluated samples and

Table 3. Evaluation of the appearance, aroma, consistency, flavor and global acceptance attributes for Greek style yoghurts formulations by untrained panelists (mean \pm SD, n = 103).

Attribute	C0	C10	C20	C30
Appearance	7.96 \pm 1.53 ^a	7.12 \pm 1.72 ^b	7.20 \pm 1.69 ^b	6.85 \pm 1.85 ^b
Aroma	6.91 \pm 1.91 ^a	6.56 \pm 1.73 ^a	6.71 \pm 1.76 ^a	6.67 \pm 1.78 ^a
Consistency	8.06 \pm 1.54 ^a	6.58 \pm 2.00 ^{bc}	6.90 \pm 1.76 ^b	6.34 \pm 2.13 ^c
Flavor	7.56 \pm 1.83 ^a	6.69 \pm 2.03 ^b	6.83 \pm 0.71 ^b	6.84 \pm 1.90 ^b
Overall acceptance	7.39 \pm 1.81 ^a	6.59 \pm 1.88 ^b	6.81 \pm 1.75 ^b	6.60 \pm 1.78 ^b

Means and standard deviation with different letters on the same line differ by the Duncan test ($P < 0.05$). C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

consequently the lowest value (6.85) for appearance attribute. This conclusion is reinforced by the findings by Abdel-Ghany & Zaki (2018), who observed an increase in yellow colour in Greek style yoghurts with different colostrum concentrations when compared to the control sample (without colostrum).

No statistical differences were observed for aroma attribute for GY with different colostrum concentrations, demonstrating that its addition did not cause changes in this parameter when comparing the samples to the control.

The sensory acceptance of the consistency attribute was reduced from 8.06 in GY without colostrum (C0) to a range of 6.34 to 6.90 in yoghurt with added colostrum. This decrease is possibly related to the increase in viscosity provided by the colostrum addition, as observed by some researchers. For example, Mouton & Aryana (2015) noticed an increase in the viscosity of ice cream enriched with colostrum. A similar fact was observed by Ayar et al. (2016) in yoghurt and kefir compositions, as well as by Abdel-Ghany & Zaki (2018) in yoghurt. Considering that GY is a product with a firmer consistency (Magalhães & Della Torre, 2018), the viscosity acquired from the colostrum addition may have influenced the reduced consistency acceptance attribute by the panelists. The C20 sample presented the best performance from the samples with colostrum added in the present work, although there was no significant difference respect to the average obtained for the C10 sample.

The values attributed for the sensory evaluation of the flavour attribute ranged from 7.56 (I liked it regularly) for the C0 sample, to values of 6.69 (slightly liked it) for the C10 sample.

The overall acceptance of all colostrum-added yoghurts was inferior to the control sample. Perhaps the addition of flavours could be favorable to the enhancement of consumer's acceptance of yoghurts with colostrum.

For the acceptability index (AI) calculation, according to Dutcosky (2007) a product is classified as having good acceptability when its attributes reach an AI greater than 70%. Therefore, it can be said that all formulations evaluated herein achieved good acceptability for all the evaluated attributes (Table 4). The good acceptance of the panelists in relation to the Greek style yoghurts in the present study reinforces the results found by Saalfeld et al. (2012). The authors reported good acceptance in milk drinks enriched with colostrum silage flavoured with strawberries and chocolate.

Table 5 shows the results obtained on the purchase intention test. The C20 GY showed the best results for the purchase intention among the yoghurts with the colostrum addition: approximately 46% of the panelists declared that they would buy that yoghurt.

The performance of yoghurt with 20% of added colostrum in the purchase intention may be related to the product consistency, which in the sensory analysis reached the best scores among the samples with colostrum added. The consistency of Greek style yoghurts is an important attribute in their acceptance. Thick, dense and firm textures are preferred for this type of product (Desai et al., 2013).

The percentage of panelists who declared they would buy the control yoghurt was 1.78 times higher than those who said

Table 4. Acceptability Index (AI) of Greek style yoghurts with different bovine colostrum additions.

Attribute	C0	C10	C20	C30
Appearance	88.4%	79.1%	80.0%	76.1%
Aroma	76.8%	72.9%	74.6%	74.1%
Consistency	89.6%	73.1%	76.7%	70.4%
Flavor	84.0%	74.3%	75.9%	76.0%
Overall acceptance	82.1%	73.2%	75.7%	73.3%

C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

Table 5. Purchase intention of Greek style yoghurts with different bovine colostrum additions.

	C0	C10	C20	C30
I would not buy it	10.68%	24.27%	14.56%	28.16%
Maybe I would buy it	18.45%	35.92%	39.81%	32.03%
I would buy it	70.87%	39.81%	45.63%	39.81%

C0 (control yoghurt); C10 (10% colostrum yoghurt); C20 (20% colostrum yoghurt); C30 (30% colostrum yoghurt).

they would buy yoghurt with 10 and 30% added colostrum, and 1.55 times higher than the percentage of people who would buy 20% colostrum-added yoghurt. These results reiterate the need to improve the formulations with colostrum added so that they achieve better scores in sensory analysis and greater purchase intention.

The evaluation of additional consumer sensory methods based in sensory perception is an important aspect of the food research and assists in understanding the behavior of the consumers (Soares et al., 2017). Consumer based methodologies are gaining popularity in the sensory and consumer science. The findings are relevant to improve the processing of product taking in account the consumer perception as well as to develop efficient marketing strategies of commercialization (Soares et al., 2020).

Preferred attribute elicitation (PAE) is a consumer-based methodology which can be performed in one session (70 min) and resulted in suitable reliability with a low number of consumers ($n = 16-21$). In this test, few consumers are asked to determine, in a consensus, the attributes that describe the samples, and rank these attributes by their importance for the liking of the products. PAE methodology can be used to characterize sensorially and to determine the consumers' acceptance of functional yoghurts and does not need training (Grygorczyk et al., 2013; Costa et al., 2020).

4 Conclusion

The bovine colostrum addition in producing Greek style yoghurts results in a greater amount of fat and protein in the product. Formulations with the addition of different colostrum percentages achieved good sensory acceptability, although they performed less than the control sample. The use of flavourings can be an alternative to increase the acceptance of Greek style yoghurts developed with colostrum.

The results of the present study suggest that the use of colostrum in producing Greek style yoghurts can reach prominence

in the dairy market, adding economic and nutritional value to the final product. Additional studies should seek to describe the functional properties of these new products. The colostrum fermentation process may be able to promote the formation of several compounds with beneficial effects on the human organism, in addition to those naturally present in bovine colostrum.

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