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# Influence of the addition of *Lactobacillus acidophilus* La-05, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and inulin on the technological, physicochemical, microbiological and sensory features of creamy goat cheese

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The effects of the addition of Lactobacillus acidophilus LA-05, Bifidobacterium animalis subsp. lactis BB-12 and inulin on the quality characteristics of creamy goat cheese during refrigerated storage were evaluated. The manufactured cheeses included the addition of starter culture (Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris - R-704) (CC); starter culture, L. acidophilus LA-05 and inulin (CLA); starter culture, B. lactis BB-12 and inulin (CBB); or starter culture, L. acidophilus LA-05, B. lactis BB-12 and inulin (CLB). In the synbiotic cheeses (CLA, CBB and CLB), the counts of L. acidophilus LA-05 and *B. lactis* BB-12 were greater than  $6\log$  CFU g<sup>-1</sup>, the amount of inulin was greater than 6 g per 100 g, and the firmness was reduced. The cheeses evaluated had high brightness values ( $L^*$ ), with a predominance of yellow (b\*). CC had higher contents of proteins, lipids and minerals compared to the other cheeses. There was a decrease in the amount of short-chain fatty acids (SCFAs) and an increase of medium-chain (MCFAs) and long-chain fatty acids (LCFAs) in the synbiotic cheeses compared to CC. The amount of conjugated linoleic acid increased in CLA, CBB and CLB. The highest depth of proteolysis and the greatest changes in the release of free amino acids were found in CLB. The addition of inulin and probiotics, alone or in co-culture, did not affect the cheese acceptance. Inulin and probiotics can be used together for the production of creamy goat cheese without negatively affecting the general guality characteristics of the product, and to add value because of its synbiotic potential.

# Introduction

Functional foods promote not only basic nutrition but also individuals' well-being.<sup>1</sup> Due to these characteristics and the

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consumer demand for products containing components with functional properties such as probiotic cultures and prebiotic ingredients, companies and researchers have become increasingly interested in developing new products that meet this emerging consumption demand.<sup>2</sup>

With regard to dairy products, goat milk has received special attention for its special nutritional and functional features, such as the presence of lactose-derived oligosaccharides and high amounts of conjugated linoleic acid (CLA), short-chain fatty acids (SCFAs), vitamins (A and complex B) and calcium.<sup>3–5</sup> Goat milk and its derivatives, in addition to their nutritional and functional characteristics, may serve as a suitable matrix for carrying probiotic microorganisms and prebiotic ingredients, providing increased nutritional and technological quality.<sup>6</sup>

Probiotics are defined as cultures from one or more species of microorganisms that provide health benefits when ingested by animals or humans. The probiotics most commonly studied

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for the development of functional foods are *Lactobacillus* and *Bifidobacterium*.<sup>7</sup> Prebiotics are characterized as non-digestible food components that reach the colon, selectively stimulating the proliferation or activity of beneficial bacteria, causing a decrease in the population of pathogenic and putrefactive bacteria.<sup>8</sup> Inulin is a prebiotic that can improve the sensory and technological characteristics of food, such as flavour, texture and moisture, in addition to its previously recognized prebiotic effects.<sup>6</sup> Due to its gelling properties, inulin has been used as a prebiotic ingredient in yogurts, dairy beverages and various types of cheeses,<sup>9</sup> adding functional aspects and better acceptance to these products.<sup>10</sup>

Creamy cheese is a fresh cheese characterized by having a thin and creamy consistency, soft and slightly sour flavor, being acidified by starter mesophilic cultures (*e.g.*, *Lactococcus* and *Leuconostoc*) and obtained from curd homogenization. This cheese can be consumed in natural form or added flavorings. Creamy cheese can be classified into two major types, considering the initial fat content and the final composition: double cream, with at least 9–11% initial fat; and single cream, with 4–5% initial fat.<sup>11</sup>

Creamy cheese made from goat milk has particular characteristics that make it a potential matrix for carrying probiotics, such as the lack of high-temperature heating of ingredients during processing and the semisolid structure that protects probiotic cultures during storage. In addition, its composition contains nutrients necessary for the growth of probiotic cultures and a slightly acidic pH, favouring the growth of lactic acid bacteria.<sup>11</sup> However, few studies have evaluated goat milk cheeses as matrices to serve as a vehicle for probiotic cultures,<sup>12–16</sup> or studied the effect of the addition of inulin on the behaviour of these microorganisms during storage.

Considering these aspects, this study evaluated the effects of the addition of inulin and probiotic cultures of *L. acidophilus* LA-05 and *B. lactis* BB-12, alone or in combination, on the technological, physicochemical, microbiological and sensory features of creamy goat cheese during storage.

# Materials and methods

# **Raw materials**

Milk used in cheese manufacture was obtained from goats of native breeds belonging to the Experimental Station of the Centre for the Training of Technologists (Federal University of Paraiba, Bananeiras, Brazil), and was stored under refrigeration ( $7 \pm 1$  °C for a maximum of 1 day) until the time of cheese processing. The starter culture consisted of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* R-704 (batch 2937721); cultures of probiotics *L. acidophilus* LA-05 (batch 2914230) and *B. lactis* BB-12 (batch 2280202) and rennet were purchased from Chris. Hansen® (Valinhos, São Paulo, Brazil). Inulin (Orafti® GR inulin) was obtained from BENEO Orafti® (Tienem, Belgium), calcium chloride (CaCl<sub>2</sub>) P.A. was obtained from FMaia Ltd® (Cotia, São Paulo, Brazil) and xanthan gum (food additive number E 322) was obtained from Gastronomy Lab (Goias, Brazil).

## Development of creamy goat cheese

Four different types of creamy goat cheeses were produced as follows: a control cheese with added mesophilic conventional starter culture composed of *L. lactis* and *L. cremoris* R-704 (CC); a cheese containing starter culture and *L. acidophilus* LA-05 in co-culture and 8 g per 100 g inulin (CLA); a cheese containing starter microorganisms and *B. lactis* (BB-12) in co-culture and 8 g per 100 g inulin (CBB); and a cheese containing starter microorganisms and *L. acidophilus* LA-05 and *B. lactis* BB-12 in co-culture and 8 g per 100 g of inulin (CLB) (Fig. 1).

The creamy cheeses were prepared following the procedure described by Alves *et al.*<sup>17</sup> and the addition of inulin was performed according to Araújo *et al.*<sup>18</sup> Fresh cheese was prepared using 10 L of pasteurized and homogenized goat milk (65 °C/ 30 min). Lactic acid bacteria (starter and probiotic) were added at a ratio of 0.06 g L<sup>-1</sup> for the starter culture in CC; and 0.1 g L<sup>-1</sup> of probiotic cultures in CLA, CBB and CLB, after heating the milk to 37 °C. Calcium chloride (0.25 g L<sup>-1</sup>) was added simultaneously to the cultures. Commercial rennet powder (Ha-la, Chris. Hansen®, Valinhos, São Paulo, Brazil), containing 88–92% bovine pepsin + 8–12.5% bovine chymosin, was added (50 mg L<sup>-1</sup>) when the milk pH reached 6.3–6.4. The mixture was then held at room temperature (30 ± 2 °C) for approximately 18 h (Fig. 1).

After milk fermentation and coagulation, the curd was cut into cubes using a lyre, followed by syneresis under refrigeration (7  $\pm$  1 °C) for 18 hours. Subsequently, the different creamy cheeses (CC, CLA, CBB and CLB) were prepared with the addition of salt (NaCl, 0.8 g per 100 g) and xanthan gum (0.5 g per 100 g). Only CLA, CBB and CLB had added inulin (8 g per 100 g).<sup>18</sup> Finally, the curd was homogenized, and the final product dispensed in individual glass jars containing 40 g of each cheese, hermetically sealed and stored under refrigeration (7  $\pm$  1 °C).

The creamy cheeses were processed in triplicate; the pH and acidity were analysed on the  $1^{st}$ ,  $7^{th}$ ,  $14^{th}$  and  $21^{st}$  days of storage, and the other analyses were carried out on the  $7^{th}$  and  $21^{st}$  days of storage.

## Characterization of creamy goat cheeses

**Microbiological analyses, viability of probiotics and quantification of inulin.** The hygienic-sanitary quality of the prepared cheeses was assessed by counting the total and faecal coliforms and coagulase-positive *Staphylococcus*, and verifying the presence/absence detection of *Salmonella* sp., using the procedures described by APHA.<sup>19</sup>

For the counts of lactic acid bacteria forming the starter culture, M17 agar (Difco®) supplemented (10 g per 100 ml) with a lactose solution (5 g per 100 ml) (Synth®) was incubated at 30 ± 1 °C for 48 hours under aerobic conditions (Anaerobic System Anaerogen, Oxoid®).<sup>20</sup> For the counts of *L. acidophilus* LA-05, MRS agar with added clindamycin (0.5 ppm) (Sigma-Aldrich® C5269, Missouri, USA) was incubated at 37 ± 1 °C for



Fig. 1 Flowchart of goat creamy cheese processing with and without addition of inulin and/or probiotics on the 7<sup>th</sup> and 21<sup>st</sup> days of storage.

72 hours under anaerobic conditions (Anaerobic System Anaerogen, Oxoid®).<sup>20</sup> For the counts of *B. lactis* Bb-12, TOS propionate agar (Merck®, New Jersey, USA) supplemented with lithium mupirocin (3 g L<sup>-1</sup>) (Sigma-Aldrich®, Missouri, USA) was incubated at  $37 \pm 1 \, ^{\circ}$ C for 72 hours under anaerobic conditions (Anaerobic System Anaerogen, Oxoid®).<sup>21</sup> The limit of detection in *L. acidophilus* and *B. lactis* count analysis was 2log CFU g<sup>-1</sup>.

The inulin content in cheeses was enzymatically determined using a Megazyme Fructan HK Assay kit® (Megazyme Inc.®, Wicklow, Ireland), according to the manufacturer's instructions.

**Technological analyses.** The yield of each creamy cheese was expressed (g cheese  $\times$  10 L<sup>-1</sup> milk) as the fresh cheese weight, in grams, obtained from 10 L of goat milk used for production.<sup>22</sup>

To determine the water holding capacity (WHC), the jar containing each creamy cheese was opened and the exudate whey was removed before analysis. Creamy cheese (approximately 20 grams) was weighed and centrifuged (13500g, 30 minutes, 10 °C). The supernatant fluid was drained for

10 minutes and weighed. The WHC was expressed as g water per 100 g of sample.<sup>23</sup>

The instrumental texture (hardness, cohesiveness, adhesiveness, elasticity and gumminess) of cheese samples was determined by a double compression test using an RT-XT2® texturometer (Stable Micro Systems®, Haslemere, UK) and an acrylic cylinder device with a diameter of 25 mm, a compression of 1 cm and a speed of 1 mm s<sup>-1</sup>. Samples were portioned into cylindrical containers with a height and diameter of 2 cm and 5 cm, respectively, and a temperature of  $10 \pm 1$  °C, being removed from the refrigerator shortly before the test. Data were collected using the Texture Expert software for Windows®, version 1.20 (Stable Micro Systems®).<sup>24</sup>

Instrumental colour determination was performed using a Minolta colorimeter, model CR300® (New Jersey, USA), and the CIELAB system.<sup>25</sup> Coordinates defined by  $L^*$  (lightness),  $a^*$  [green chromaticity (–)/red (+)] and  $b^*$  [blue chromaticity (–)/yellow (+)] were analysed. Measurements were performed in triplicate on a previously calibrated apparatus immediately after removal from the package.

#### Physicochemical analyses

Water activity  $(a_w)$ , pH, acidity, chemical composition and organic acids. The  $a_w$  was determined using an Aqualab® apparatus (model CX-2 Water Activity System, Washington, USA), according to the manufacturer's instructions.<sup>26</sup>

The pH was determined using a previously calibrated pH meter (model Tec-2 Tecnal®) that was operated according to the manufacturer's instructions. The acidity was determined by titration with a 0.1 N sodium hydroxide solution (AOAC 920.124) according to AOAC.<sup>27</sup>

The moisture (AOAC 925.09), ashes (AOAC 935.30), lipids (Gerber; AOAC 2000.18), crude protein (AOAC 939.02) and lactose (AOAC 920.82) analyses were performed using standard procedures.<sup>27</sup>

The production of lactic, citric, acetic and succinic acids was determined using high-performance liquid chromatography (HPLC) according to Bruno, Lankaputhra and Shah.<sup>28</sup> Briefly, 2 g of each creamy cheese were weighed and added to 10 mL of an acidic solution prepared with 95–97% of 13 mM sulfuric acid. Samples were homogenized in a "mixer" for 3 minutes at 18 000 rpm and then centrifuged for 10 minutes at 4000 rpm. The supernatant was filtered through #1 filter paper (V. Reis®, Portugal) and the extract was filtered through a 0.22 µm membrane filter (Orange Scientific®, Belgium) immediately before injection.

Organic acids were quantified in a single run using previously prepared calibration curves on an HPLC instrument from Merck LaChrom® (Fullerton, CA, USA) with an Aminex HPX-87X cation exchange column ( $300 \times 7.8$  mm, Bio-Rad, USA) maintained at 65 °C. The eluent flow rate ( $13 \text{ mM H}_2\text{SO}_4$ , Merck®, USA) was 0.8 ml min<sup>-1</sup>. The organic acids were detected using ultra-violet light (L-7400 UV detector; LaChrom, Merck-Hitachi®, Fullerton, CA, USA) at an absorbance of 220 nm. Data were obtained by using the D7000 interface (LaChrom, Merck-Hitachi®, Fullerton, CA, USA) and analysed using the HPLC System Manager® Software 3.1.1 (Merck-Hitachi®, Fullerton, CA, USA).

Fatty acid profile, relationships between fatty acids, and CLA. Initially, the fat was extracted from each cheese sample according to the procedure described by Folch et al.<sup>29</sup> followed by the preparation of methyl esters.<sup>30</sup> Transmethylated samples were then analysed by gas chromatography for the determination of total fatty acids. CLA was measured using reference standards to determine the recovery and correction factors for individual fatty acids (Supelco® 37 FAME Mix, Sigma-Aldrich, Bellefonte, PA, USA). Individual fatty acids were identified and quantified by comparison of retention times and peak areas with their respective standards. Data were collected using the Galaxie Chromatography Data System Software® and expressed as the percentage (% w/w) of total fatty acid methyl esters (FAMEs). The proportion of CLA (isomer C18:2 cis-9, trans-11) in the samples was determined using the procedure described by Glasser et al.31

Fatty acids were grouped into SCFAs (C4:0 to C9:0), medium-chain fatty acids (MCFAs; C10:0 to C15:1), long-chain

fatty acids (LCFAs; C16:0 to C24:0), saturated fatty acids (SFAs), monounsaturated fatty acids (MFAs) and polyunsaturated fatty acids (PUFAs).

**Proteolysis and free amino acids.** Soluble nitrogen (NS) in 1.2 g L<sup>-1</sup> trichloroacetic acid (TCA) and cheese with pH 4.6 was determined using the micro-Kjeldahl method.<sup>27</sup> Proteolysis was evaluated based on the proteolysis extension index (%) = (NS at pH 4.6)/TN (total nitrogen) × 100 and the proteolysis depth index (%) = (NS at TCA) × 100.<sup>32</sup>

Free amino acids were evaluated in cheese samples through extraction by orbital shaking for 60 min with 0.1 M hydrochloric acid (g mL<sup>-1</sup>), followed by derivatization according to the procedures described by White, Hart and Fry<sup>33</sup> and Hagen, Frost and Augustin.<sup>34</sup> An aliquot of 50 µL of this diluted solution was injected into a Shimadzu high-efficiency liquid chromatograph with a C18 guard column  $(4 \times 3.0 \text{ mm})$  and a Shim-Pack CLC G-ODS analytical column (Luna C18, 100 Å, 5 µM, 250 × 4.6 mm, 00G-4252-EQ). The flow of eluents (mobile phase) was 1 ml min<sup>-1</sup> at 50 °C, and a diode array detector (DAD) was used with detection at 254 nm. Chromatographic separation occurred at a constant flow rate of 1 mL min<sup>-1</sup> at 35 °C. The chromatographic run lasted 45 min, and the results were expressed as mg of amino acid per 100 g of cheese. Quantification was performed by adding α-aminobutyric acid as an internal standard.

#### Sensory analysis

A sensory evaluation of the cheeses was carried out on the 7<sup>th</sup> and 21<sup>st</sup> day of refrigerated storage, considering the time required to achieve balance of the biochemical components that interfere with the cheese flavour ("maturation"). Only cheeses that were within the microbiological standards recommended by specific legislations underwent analysis.<sup>35</sup> The sensory analysis received approval from an Ethics Committee (Protocol No. 111523).

Acceptance and purchase intention tests were performed following the criteria established by Amerine, Pangborn and Roessler.<sup>36</sup> Briefly, a panel consisting of 100 untrained tasters was selected based on habits and interest in consuming creamy cheese. The panel consisted of 71 female and 29 male subjects aged 18–45 years with no health problems or disabilities that would compromise the sensory evaluation of products. The panellists analysed different cheese samples in a monadic way.<sup>37</sup>

In the acceptance test, the sensory attributes of appearance, colour, aroma, flavour, texture and overall acceptance were evaluated. The panellists assigned values in a structured 9-point hedonistic scale (1 = disliked extremely, 5 = neither liked nor disliked, 9 = liked very much). In the purchase intention test, values were assigned in a structured 5-point scale (1 = certainly would not buy, 3 = may buy/may not buy, 5 = certainly buy). The analysed samples were considered accepted with an average score >5.0 (equivalent to the hedonic term "neither liked nor disliked").<sup>38</sup>

During the tests, the samples were served in random order. Analyses were conducted in individual cabins with controlled

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temperature and lighting, and the samples were served at approximately 10 °C in white plastic cups coded with a random 3-digit number and accompanied by mineral water and salty biscuits.

## Statistical analyses

Data analysis was performed using descriptive (mean and standard deviation) and inferential statistics tests (ANOVA followed by Tukey's test and Student's *t*-test) to determine the differences ( $p \le 0.05$ ) between treatments. Principal component analysis (PCA) was performed to provide a graphical representation of the significant free amino acids<sup>39</sup> using the Statistical Analysis System (SAS), version 6.2 described by SAS.<sup>40</sup>

# Results and discussion

# Microbiological analysis, viability of probiotics and inulin quantification

The different types of manufactured goat creamy cheeses presented satisfactory hygienic-sanitary conditions, with the total coliform, faecal coliform and coagulase-positive *Staphylococcus* counts during storage below the maximum limits established by current legislation.<sup>35</sup> In addition, there was no detection of *Salmonella* spp.

The counts of *L. acidophilus* LA-5 on the 7<sup>th</sup> and 21<sup>st</sup> days of storage were 6.93 (±0.1) and 6.90 (±0.3) log CFU g<sup>-1</sup> in CLA and 6.5 (±0.1) and 6.49 (±0.1) log CFU g<sup>-1</sup> in CLB, respectively. For *B. lactis* BB-12, the counts on the 7<sup>th</sup> and 21<sup>st</sup> days of storage were 7.85 (±0.1) and 7.76 (±0.1) log CFU g<sup>-1</sup> in CBB and 7.84 (±0.1) and 7.77 (±0.1) log CFU g<sup>-1</sup> in CLB, respectively.

The initial counts of *L. acidophilus* LA-05 and *B. lactis* BB-12 were higher ( $p \le 0.05$ ) on the 7<sup>th</sup> day compared to the 21<sup>st</sup> day of storage. However, the reduction in counts during this period was never greater than 0.15log CFU g<sup>-1</sup>.

The minimum count of probiotics to obtain health benefits is 6-7log CFU of viable cells per gram of food product.<sup>41,42</sup> Based on this recommended count, the goat creamy cheeses assessed in this study had sufficient counts of probiotics over 21 days of storage to provide benefits to consumers' health. The counts of L. acidophilus and B. lactis were always <2log CFU g<sup>-1</sup> in CC over the evaluated storage periods. The average amount of inulin was 7.50 g per 100 g  $(\pm 0.01)$  in CLA; 7.55 g per 100 g (±0.02) in CBB and 7.00 g per 100 g (±0.01) CLB on the 7<sup>th</sup> day of storage and 6.85 g per 100 g ( $\pm 0.04$ ) in CLA; 6.70 g per 100 g (±0.01) in CBB and 6.67 g per 100 g  $(\pm 0.01)$  in CLB on the 21<sup>st</sup> day of storage. The Brazilian standard establishes a creamy cheese portion as being 30  $g_1^{43}$ which should provide an amount of at least 2 g of inulin. In the present study, the amount of fructans was above 6 g per 100 g in creamy goat cheeses with added probiotic cultures and inulin throughout the storage period, which is sufficient to provide prebiotic potential, assuming a daily consumption of 100 g. Beneficial changes in intestinal flora composition were observed with a daily intake of 100 g of food product containing 5-20 g of inulin, usually with administration for a

period of 15 days. Thus, to stimulate the proliferation of beneficial bacteria in the colon, daily doses of 4–5 g of inulin should be effective.  $^{44-47}$ 

#### **Technological analysis**

The WRC values ranged from 34.4 to 48.39% (Table 1). On the 7<sup>th</sup> day of storage, the addition of inulin and probiotics to CLA, CBB and CLB slightly increased ( $p \le 0.05$ ) the WRC content compared to the CC, which can be explained by the ability of dietary fibre, such as inulin, to interfere with the technological properties of food, including increased WHC, emulsification and gel formation.<sup>48</sup> Notably, the maintenance and increase of WHC provide better viscosity and consistency compared to creamy cheeses.<sup>49</sup> After 21 days of storage, the difference between the groups disappeared.

The yield of prepared goat creamy cheeses ranged from 271.07 to 364.54 g L<sup>-1</sup>, with no difference (p > 0.05) between them (Table 1). In turn, Buriti<sup>24</sup> found that the incorporation of a probiotic culture of *L. paracasei* and inulin increased the yield of probiotic creamy cheese. In fact, there was no significant difference in terms of yield between the control and synbiotic cheeses, because the yield analysis was conducted on the first day of storage of creamy cheese, just after processing, when the establishment of physical properties, such as increased water retention capacity, was not visible yet. The increase in the water retention capacity of synbiotic cheeses was measured by 7 days of storage and a significant increase in synbiotic cheese compared with the control cheeses was observed.

Regarding texture parameters, the firmness values were lower ( $p \le 0.05$ ) in cheese containing inulin, *L. acidophilus* La-05 and/or *B. lactis* BB-12 compared to CC (Table 1). This result could be explained by the interference of inulin with some texture parameters of food products, such as the improvement of consistency of cheese and ice cream.<sup>50</sup> Dietary fibres with high WHC can be used to prevent syneresis and modify the viscosity and texture of certain food products.<sup>51</sup> CLA may have shown the lowest firmness because of the high proteolytic activity and peptide production by *L. acidophilus*, likely as a result of the greater production and activity of endopeptidases.<sup>52</sup>

Between the 7<sup>th</sup> and 21<sup>st</sup> days of storage, the firmness of CLA, CBB and CLB increased ( $p \le 0.05$ ). Buriti, Cardarelli and Saad<sup>53</sup> when studying creamy cheese with added *L. paracasei* subsp. *paracasei* and inulin also observed increased firmness within the first two weeks of storage. These results may be related to the level of cheese acidification, which greatly impacts the product texture as a result of the demineralization of casein micelles.<sup>24</sup> In the present study, increased acidity over the refrigerated storage period was observed, which may have contributed to the increased firmness of cheeses due to increased whey loss (syneresis), which was evidenced by the reduced WRC observed in all cheeses between the 7<sup>th</sup> and 21<sup>st</sup> days of storage.

The adhesiveness and elasticity in CBB and CLB, cohesiveness in CBB and gumminess in CLA were reduced ( $p \le 0.05$ )

**Table 1** Mean values  $\pm$  standard deviation of the technological analysis of WHC<sup>a</sup>, yield, texture and colour of goat cheese with and without addition of inulin and/or probiotics on the 7<sup>th</sup> and 21<sup>st</sup> days of storage

		Creamy cheeses			
Trials	Days	CC	CLA	CBB	CLB
$WRC^{a}$ (g H <sub>2</sub> O per 100 g)	7	$46.46^{b_{*}} \pm 0.30$	$48.07^{a_{*}} \pm 0.54$	$47.55^{a_{*}} \pm 0.10$	$48.39^{a_{*}} \pm 0.56$
	21	$37.643^{a} \pm 4.80$	$34.44^{\mathrm{a}} \pm 2.60$	$35.10^{ m a} \pm 1.56$	$35.03^{\mathrm{a}} \pm 4.56$
Yield (g per 10 L milk)	1	$364.54^{a} \pm 69.76$	$308.41^{a} \pm 65.04$	$271.06^{a} \pm 64.45$	$303.82^{a} \pm 37.71$
Texture					
Firmness	7	$227.27^{a} \pm 2.1$	$63.08^{ m c} \pm 1.87$	$141.55^{ m b}\pm 0.04$	$161.00^{ m b} \pm 1.17$
	21	$486.59^{a_{*}} \pm 0.1$	$85.59^{c*} \pm 0.01$	$176.56^{b_{*}} \pm 0.22$	$222.44^{b} \pm 0.21$
Adhesiveness	7	$-402.14^{b_{*}} \pm 0.22$	$-167.48^{a_{*}} \pm 1.3$	$-595.59^{c*} \pm 0.23$	$-470.03^{b*} \pm 0.01$
	21	$-522.16^{b} \pm 0.11$	$-248.30^{\rm a} \pm 0.25$	$-885.73^{d} \pm 0.11$	$-644.02^{\circ} \pm 0.12$
Elasticity	7	$0.84^{a_{*}} \pm 0.07$	$0.87^{\rm a}\pm0.02$	$0.70^{b_{*}} \pm 0.14$	$0.79^{a_{*}} \pm 0.04$
-	21	$0.68^{\rm b}\pm0.14$	$0.89^{\mathrm{a}} \pm 0.02$	$0.32^{c} \pm 0.07$	$0.63^{ m b} \pm 0.14$
Cohesiveness	7	$0.50^{\rm ab}\pm0.12$	$0.55^{\rm a} \pm 0.02$	$0.37^{b*} \pm 0.11$	$0.43^{ m ab} \pm 0.04$
	21	$0.45^{\mathrm{ab}}\pm0.10$	$0.58^{\rm a}\pm0.01$	$0.18^{\rm c} \pm 0.03$	$0.40^{\rm b}\pm0.15$
Gumminess	7	$64.90^{\mathrm{a}} \pm 9.06$	$35.31^{\rm b} \pm 6.72$	$76.94^{ m a} \pm 24.54$	$68.16^{a} \pm 10.81$
	21	$77.51^{\mathrm{a}} \pm 8.87$	$50.23^{\mathrm{b}} \pm 12.53$	$90.26^{a} \pm 5.37$	$82.62^{a} \pm 8.9$
Colour					
L	7	$91.61^{a} \pm 0.59$	$90.56^{a} \pm 3.34$	$93.48^{\rm a} \pm 0.16$	$92.66^{a} \pm 0.65$
	21	$91.75^{ m b} \pm 0.49$	$94.08^{ m a} \pm 0.35$	$93.00^{\mathrm{a}} \pm 0.80$	$93.06^{a} \pm 0.04$
<i>a</i> *	7	$-3.43^{a} \pm 0.37$	$-3.41^{a} \pm 0.29$	$-3.53^{\rm a} \pm 0.03$	$-3.48^{a} \pm 0.36$
	21	$-3.63^{\rm c} \pm 0.05$	$-3.5^{bc} \pm 0.05$	$-3.34^{ab*} \pm 0.10$	$-3.25^{a} \pm 0.15$
<i>b</i> *	7	$10.63^{\mathrm{a}} \pm 0.47$	$10.35^{\mathrm{a}} \pm 1.10$	$10.68^{\mathrm{a}}\pm0.18$	$10.42^{a} \pm 0.29$
	21	$11.23^{ m ab} \pm 0.37$	$11.25^{\mathrm{a}}\pm0.20$	$10.68^{ m ab} \pm 0.26$	$10.25^{ m b} \pm 0.55$

<sup>a-c</sup>Means  $\pm$  standard deviation with different letters in the same line differ by Tukey's test ( $p \le 0.05$ ). \*Means  $\pm$  standard deviation in the same column differ from each other by Student's *t*-test. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8% inulin; and CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin. <sup>*a*</sup> WRC – water holding capacity.

between the 7<sup>th</sup> and 21<sup>st</sup> days of storage (Table 1). The differences in these texture parameters could be associated with the initial composition of the goat milk used in the preparation of products and final products themselves.<sup>54</sup>

Regarding colour, in general, the cheeses analysed had high brightness values  $(L^*)$  with a predominance of yellow  $(b^*)$  compared to the green component  $(a^*)$ , suggesting that a yellowish white colour contributed more to their colour characteristics (Table 1). The  $L^*$  parameter indicates the ability to transmit light based on a scale ranging from 0 to 100. In this sense, a higher  $L^*$  value corresponds to lighter food. Cheese made from goat milk tends to be white due to its ability to convert carotene into vitamin A and also due to its smaller-diameter fat globules compared to cow's milk,<sup>55</sup> which explains the high  $L^*$  values detected in this study.

The  $L^*$  and  $b^*$  values did not differ (p > 0.05) over the storage period (Table 1), which is in accordance with the results of studies with goat cheeses.<sup>56,57</sup> The  $a^*$  values were higher  $(p \le 0.05)$  in CBB and CLB on the 21<sup>st</sup> day of storage, probably due to the ability of probiotic bacteria to synthesize complex B vitamins, which can contribute to the production of green pigments in foods.<sup>58</sup>

#### Physicochemical analyses

 $a_{w}$ , pH, acidity, chemical composition and organic acids. The  $a_{w}$  values on the 21<sup>st</sup> day of storage (Table 2) showed no differences between the goat cheeses with added inulin, *L. acidophilus* LA-05 and/or *B. lactis* Bb-12 (Table 2).

CLA, CBB and CLB had higher acidity ( $p \le 0.05$ ) and hence lower pH values ( $p \le 0.05$ ) compared to CC (Fig. 2). Throughout the storage period, there was a decrease in pH, which is a natural process caused by the continuing production of lactic acid and other organic acids by the starter and probiotic cultures in cheeses, as demonstrated by other studies with synbiotic cheeses.<sup>18,24,41,59</sup>

Compared to CC on the 7<sup>th</sup> day of storage, CLA, CBB and CLB presented lower amounts ( $p \le 0.05$ ) of proteins, and CLA and CLB presented lower amounts of fat. The lower amounts of fat in CLB and CLA were possibly due to the addition of prebiotic ingredients to the cheese mass, changing the proportion of the chemical composition since the analysis was performed on a wet basis. This decrease could also be due to the more intense proteolysis and lipolysis caused by the activity of lactic acid bacteria used in cheese manufacture.

Araújo *et al.*<sup>18</sup> found similar results when studying synbiotic cottage cheese, which showed a reduction in fat and protein contents compared to the control cheese. In addition, a reduction ( $p \leq 0.05$ ) in ash content was observed in CLA, CBB and CLB only on the 21<sup>st</sup> day of storage compared to CC, and there was a reduction in ash content throughout the storage period. In the same storage period, there were no differences (p > 0.05) in moisture contents of different goat creamy cheeses. The low lactose contents in all the cheeses

		Creamy cheeses			
Trials	Days	CC	CLA	CBB	CLB
a <sub>w</sub> <sup>a</sup>	7	$0.98^{ m b} \pm 0.00$	$0.97^{ m b} \pm 0.00$	$0.98^{\rm b} \pm 0.00$	$0.99^{\rm a} \pm 0.00$
	21	$0.98^{\rm a}\pm0.00$	$0.97^{\rm a}\pm0.01$	$0.97^{\rm a}\pm0.01$	$0.98^{a} \pm 0.00$
Moisture (g per 100 g)	7	$76.16^{a_{*}} \pm 0.72$	$73.31^{\mathrm{b}} \pm 0.09$	$73.53^{b} \pm 0.03$	$73.53^{b} \pm 1.04$
	21	$74.03^{a} \pm 0.13$	$72.96^{\rm a} \pm 0.49$	$74.26^{a_{*}} \pm 0.05$	$73.53^{a} \pm 1.04$
Ashes (g per 100 g)	7	$1.07^{a_{*}} \pm 0.01$	$1.05^{ab*} \pm 0.00$	$1.01^{b*} \pm 0.00$	$1.05^{ab} \pm 0.02$
	21	$0.88^{\mathrm{a}}\pm0.02$	$0.70^{\rm b}\pm0.01$	$0.75^{b} \pm 0.03$	$0.72^{b} \pm 0.03$
Protein (g per 100 g)	7	$9.64^{ m a} \pm 0.11$	$7.27^{\rm c} \pm 0.00$	$7.43^{b} \pm 0.00$	$6.46^{d} \pm 0.02$
	21	$9.63^{a} \pm 0.03$	$7.71^{b*} \pm 0.01$	$7.76^{b*} \pm 0.01$	$6.37^{c} \pm 0.02$
Lipids (g per 100 g)	7	$11.50^{a_{*}} \pm 0.05$	$10.64^{c*} \pm 0.14$	$9.79^{d} \pm 0.05$	$11.18^{b_{*}} \pm 0.02$
	21	$8.81^{\mathrm{b}}\pm0.01$	$8.37^{d} \pm 0.00$	$8.95^{\mathrm{a}} \pm 0.00$	$8.76^{\circ} \pm 0.01$
Lactose (g per 100 g)	7	$6.23^{c*} \pm 0.11$	$6.84^{a_{*}} \pm 0.01$	$6.63^{b*} \pm 0.01$	$6.81^{a_{*}} \pm 0.00$
	21	$5.67^{a} \pm 0.23$	$5.62^{a} \pm 0.00$	$5.80^{ m a} \pm 0.07$	$5.61^{a} \pm 0.00$
Organic acids					
Lactic acid (g per 100 g)	7	$21.01^{ m c} \pm 0.01$	$21.92^{\rm b}\pm0.01$	$15.86^{d} \pm 0.02$	$22.97^{a} \pm 0.01$
	21	$21.40^{c*} \pm 0.01$	$22.37^{b*} \pm 0.01$	$15.55^{ m d} \pm 0.01$	$23.11^{a_{*}} \pm 0.01$
Citric acid (g per 100 g)	7	$8.69^{\rm a} \pm 0.02$	$4.83^{c*} \pm 0.01$	$4.13^{d} \pm 0.07$	$6.29^{b} \pm 0.01$
	21	$8.94^{a_{*}} \pm 0.02$	$4.69^{ m c} \pm 0.01$	$4.68^{c_{*}} \pm 0.02$	$6.71^{b_{*}} \pm 0.26$
Acetic acid (g per 100 g)	7	$0.74^{ m a} \pm 0.01$	$0.29^{\rm d} \pm 0.06$	$0.43^{ m c} \pm 0.01$	$0.53^{b} \pm 0.01$
	21	$0.97^{a_{*}} \pm 0.01$	$0.55^{c_{*}} \pm 0.02$	$0.46^{d_{*}} \pm 0.02$	$0.59^{b_{*}} \pm 0.01$
Succinic acid (g per 100 g)	7	$0.24^{ m c} \pm 0.20$	$0.34^{\mathrm{a}} \pm 0.01$	$0.17^{d_{*}} \pm 0.01$	$0.28^{\mathrm{b}} \pm 0.10$
	21	$0.25^{\rm b} \pm 0.01$	$0.34^{\rm a} \pm 0.30$	$0.14^{\rm c} \pm 0.20$	$0.34^{a_{*}} \pm 0.12$

**Table 2** Mean values  $\pm$  standard deviation of  $a_{w}$ , chemical composition and organic acids in goat creamy cheese with and without addition of inulin and/or probiotics on the 7<sup>th</sup> and 21<sup>st</sup> days of storage

<sup>a-d</sup>Means  $\pm$  standard deviation with different letters in the same line differ by Tukey's test ( $p \le 0.05$ ). \*Means  $\pm$  standard deviation in the same column differ from each other by Student's *t*-test. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. cremoris; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8% inulin and CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin. <sup>*a*</sup>  $a_w$  – water activity.

analyzed on the 21<sup>st</sup> day of storage suggest high consumption of lactose by the starter and probiotic cultures over time, which was accompanied by an increase in lactic acid production.

Lactic acid was the organic acid identified in larger amounts in all goat creamy cheeses, with values ranging from 15.55 to 23.11 g per 100 g (Table 2). The lactic acid content was higher ( $p \le 0.05$ ) in CLB on the 7<sup>th</sup> and 21<sup>st</sup> days of storage compared to CC, CLA and CBB, which may be a result of *L. acidophilus* LA-05 and *B. lactis* BB-12 when applied in coculture, which under interaction can be stimulated to produce more lactic acid.

The citric acid content was lower ( $p \le 0.05$ ) in CLA, CBB and CLB compared to CC, which may be related to the fermentative action of lactic acid bacteria, especially L. acidophilus (Table 2). Thomas<sup>60</sup> and Ong and Shah<sup>61</sup> observed a reduction of citrate in cheddar cheese as a result of Lactobacillus metabolism. Citrate in milk is metabolized by lactic acid bacteria into flavour compounds such as acetic acid, acetaldehyde and diacetyl.<sup>62,63</sup> However, the citric acid content was higher in CLB as compared to CLA and CBB at 7 and 21 days, which means that the bacteria in the consortium have used to a lesser extent the citric acid present in the milk. This could have occurred due to the interaction between probiotic microorganisms that are in co-culture, with the consequent reduction of microbial activity of the combined strains towards the use of citric acid. Throughout the storage period, the citric acid content increased ( $p \le 0.05$ ) in CC, CBB and CLB possibly

due to the citrate metabolism that is involved in the citric acid cycle, acting as both a product and a substrate for microbial activity.<sup>64</sup>

The amounts of acetic acid were lower  $(p \le 0.05)$  in CLA, CBB and CLB compared to CC (Table 2). High acetic acid production may be undesirable in fresh cheese because of its negative impact on flavour.<sup>65</sup> Accordingly, the reduction in the amounts of acetic acid in creamy cheeses with added inulin, L. acidophilus LA-05 and/or B. lactis BB-12 is a desirable feature for maintaining the sensory characteristics of the product during storage. Although differences have been found in acetic acid content among different goat creamy cheeses, the amounts of this acid increased ( $p \le 0.05$ ) in all cheeses over the assessed storage time. Considering that the presence of acetic acid in dairy products is a result of the heterofermentative metabolism of non-starter bacteria,<sup>66</sup> the increase of this compound during storage can be explained by the presence of the probiotic cultures in CLA, CBB and CLB. In addition, the proteolytic action of the added probiotic cultures may be related to the increased acetic acid content in cheeses because free amino acids (especially alanine and serine) may act as precursors for the formation of this organic acid in microbial metabolism.67

Small amounts of succinic acids were also identified in creamy cheeses (Table 2). The production of this acid in cheese is probably due to the action of non-starter lactic acid bacteria. Importantly, *Lactobacillus* is capable of producing citric acid, including *L. acidophilus*.<sup>68</sup>



Fig. 2 Mean pH and acidity in lactic acid (%) values of goat creamy cheeses with and without addition of inulin and/or probiotics on the 7th and 21st days of storage. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8% inulin.

In general, CBB contained the smallest amounts ( $p \le 0.05$ ) of organic acids on the 21<sup>st</sup> day of storage (Table 2), which may be related to the inhibition of the heterofermentative characteristics of *B. lactis* resulting from the combination with the starter culture, as was previously reported for fermented skim milk with added *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. lactis*, *Streptococcus thermophilus*, maltodextrin, oligofructose and polydextrose.<sup>69</sup> The results of this study show that a smaller amount of acetic acid in creamy cheeses should be related to a lower succinic acid content and a higher lactic acid content, which is in agreement with previous studies on dairy products with added probiotic cultures and/or synbiotic ingredients.<sup>61,69,70</sup>

**Fatty acid profile, their relationships, and CLA.** The addition of the probiotic cultures and inulin reduced the amount of SCFAs in CLA, CBB and CLB only on the 7<sup>th</sup> day of storage (Table 3), which could lead to changes in the sensory quality of cheeses since SCFAs are associated with more significant

aromatic changes in dairy products.<sup>71</sup> Among the SCFAs, caprylic acid (C8:0) has probably most contributed to the decrease in SCFA amounts in cheese containing inulin, L. acidophilus LA-05 and/or B. lactis BB-12. This result confirms that caprylic acid, together with capric acid (C10:0), imparted a goat-type taste, which is a refusal factor of goat dairy products when pronounced. The MCFA contents were higher ( $p \le 0.05$ ) in cheese containing inulin, L. acidophilus LA-05 and/or B. lactis BB-12 only on the 7<sup>th</sup> day of storage, and the LCFA contents were greater ( $p \le 0.05$ ) in only CLB compared to CC. The LCFA omega-3 eicosatrienoic acid (C20:3 ω3), omega-6 dihomo- $\gamma$ -linolenic acid (C20:3  $\omega$ 6),  $\gamma$ -linolenic acid (C18:3  $\omega$ 6) and arachidonic acid (C20:4  $\omega$ 6) contents were higher ( $p \leq$ 0.05) in cheeses with added inulin, B. lactis and/or L. acidophilus (CBB and CLB) compared to CC on the 7<sup>th</sup> day of storage (Table 3). Rodrigues et al.<sup>72</sup> evaluated synbiotic cheese made with cow's milk and observed that the addition of probiotic cultures of L. casei-01 and B. lactis B94 increased the amount of free fatty acids during storage.

Most of the fatty acids determined in different types of creamy goat cheeses were represented by their saturated portion (SFAs) in relation to MUFAs and PUFAs, but only on the 7<sup>th</sup> day of storage (Table 3). The content of SFAs increased  $(p \le 0.05)$  in CLA, CBB and CLB on the 7<sup>th</sup> day of storage. Similarly, Yadav *et al.*<sup>73</sup> found that the addition of probiotics to the Indian fermented milk dahi increased the saturated portion of the product due to the lipolytic activity of the lactic acid bacteria used - L. acidophilus, producing higher amounts of butyric acid. The MUFA and PUFA contents on the 7<sup>th</sup> day of storage remained unchanged in CLA, CBB and CLB. Similarly, Ekinci et al.74 did not observe any influence of different probiotic cultures on the oleic acid content in the most representative PUFA in cheeses. The main reason could be related to the short storage times of the products studied, namely fermented cream - 1 day storage and synbiotic cream cheese - 7 days storage. On the other hand Rodrigues et al.72 observed a decrease in oleic acid in cheese inoculated with L. casei stored for 60 days. Studies have proven that oleic acid can be used as a substrate for the production of CLA isomers.<sup>75</sup>

The CLA content (C18:2 C9t11) ranged from 3.05 mg  $g^{-1}$  in CC to 7.69 mg  $g^{-1}$  in CLB (Table 3). The CLA content was detected in higher amounts ( $p \le 0.05$ ) in CLA, CBB and CLB on the 7<sup>th</sup> and 21<sup>st</sup> days of storage. Similarly, studies have shown that the addition of the probiotics Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus and L. acidophilus increased the CLA content in buffalo cheese, yogurts with added fructooligosaccharides (FOS), fermented milk, dahi and cream milk.73,74,76-78 Species belonging to the genera Lactobacillus and Bifidobacterium have high lipolytic capacities, producing CLA through the isomerisation of linoleic acid released during milk fat lipolysis by the action of microbial esterases.<sup>73,76,77,79,80</sup> CLB presented a higher CLA content on the 7<sup>th</sup> day of storage in relation to CC, CLA and CBB, possibly due to the higher production of microbial isomerase from the presence of a co-culture (starter and two species of probiotics).<sup>81</sup> Rodrigues et al.<sup>72</sup> observed an

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Table 3	Mean values ± standard deviation of fatty acids, relationships between fatty acids, and CLA <sup>c</sup> in goat creamy cheese with or without addition
of inulin	and/or probiotics on the 7 <sup>th</sup> and 21 <sup>st</sup> days of storage

		Creamy cheeses					
Fatty acid (mg $g^{-1}$ )	Days	CC	CLA	CBB	CLB		
Short chain							
C8:0	7	$9.36^{a_{*}} \pm 0.36$	$1.42^{b_{*}} \pm 0.38$	$1.12^{b_{*}} \pm 0.01$	$1.38^{b_{*}} \pm 0.31$		
	21	$0.19^{\mathrm{a}} \pm 0.10$	$0.13^{\mathrm{a}} \pm 0.04$	$0.15^{\mathrm{a}}\pm0.08$	$0.08^{\rm a}\pm0.06$		
C10:0	7	$37.21^{b} \pm 0.21$	$32.95^{bc} \pm 3.43$	$28.13^{\circ} \pm 0.40$	$48.11^{a_{*}} \pm 0.08$		
	21	$37.37^{\rm a} \pm 0.09$	$35.42^{a} \pm 3.83$	$42.40^{a*} \pm 4.28$	$39.17^{a} \pm 0.43$		
Medium chain		9	9	9			
C11:0	7	$1.33^{\circ} \pm 0.26$	$2.51^{a*} \pm 0.20$	$1.73^{\circ} \pm 0.02$	$2.00^{\circ} \pm 0.51$		
C12.0	21	$1.56^{\circ} \pm 0.33$	$1.32^{\circ} \pm 0.15$	$1./8^{a} \pm 0.18$	$1.65^{\circ} \pm 0.01$		
C12:0	21	$17.21 \pm 0.05$ $14.02^{b} \pm 4.05$	$20.81 \pm 0.78$ 20.71 <sup>ab</sup> + 0.86	$21.75 \pm 0.31$ $22.72^{a} \pm 1.27$	$23.33 \pm 0.43$ $22.20^{ab} \pm 1.52$		
C13:0	21	$14.02 \pm 4.03$ 0.95 <sup>b</sup> + 0.01	$104^{b} + 0.03$	$25.72 \pm 1.27$ 1 $94^{a*} \pm 0.02$	$23.30 \pm 1.33$ $1.51^{ab} \pm 0.38$		
013.0	21	$1.47^{a} \pm 0.56$	$1.04 \pm 0.03$ $1.13^{a} \pm 0.15$	$1.94 \pm 0.02$ $1.23^{a} \pm 0.08$	$1.01 \pm 0.00$ $1.19^{a} \pm 0.07$		
C14:0	7	$35.62^{\circ} + 2.28$	$41.86^{\circ} + 1.35$	$69.33^{b} + 0.42$	$86.86^{a} + 3.32$		
	21	$67.41^{b_*} \pm 1.16$	$66.49^{b} \pm 2.41$	$68.87^{\rm b} \pm 0.23$	$75.28^{a} \pm 1.31$		
C14:1	7	$3.57^{\rm a} \pm 0.38$	$3.57^{a_{\star}} \pm 0.13$	$4.13^{\mathrm{a}} \pm 0.05$	$4.82^{\mathrm{a}} \pm 1.05$		
	21	$1.46^{\rm b}\pm0.58$	$1.13^{ m b} \pm 0.12$	$4.47^{ m a} \pm 0.49$	$3.44^{\mathrm{a}}\pm0.38$		
C15:0	7	$7.24^{\rm b} \pm 0.15$	$7.71^{a} \pm 0.19$	$8.81^{ m ab} \pm 0.12$	$9.24^{a} \pm 0.78$		
	21	$8.47^{a} \pm 0.45$	$8.98^{a} \pm 0.54$	$8.51^{a} \pm 0.46$	$8.47^{a} \pm 0.47$		
C15:1	7	$2.30^{\circ} \pm 0.21$	$2.56^{\text{bc}} \pm 0.00$	$3.65^{a_{*}} \pm 0.05$	$2.95^{\text{D}} \pm 0.06$		
	21	$2.43^{a} \pm 0.33$	$2.85^{a} \pm 0.51$	$2.78^{a} \pm 0.24$	$2.73^{a} \pm 0.10$		
C16:0	7	$150.38^{\circ} \pm 0.24$	$176.12^{b} \pm 3.22$	$177.72^{5} \pm 2.56$	$195.71^{a} \pm 0.35$		
016.1	21	$162.69^{\circ*} \pm 0.15$	$1/9.16^{\circ} \pm 0.28$	$195.61^{a*} \pm 2.66$	$198.69^{a*} \pm 0.75$		
C16:1	/	$9.58^{\circ} \pm 0.66$ 10 57 <sup>a</sup> + 1.47	$10.76 \pm 0.04$ 11.99 <sup>a</sup> + 1.02	$11.51^{\circ} \pm 0.16$ 11.70 <sup>a</sup> + 0.62	$11.96^{\circ} \pm 0.00$ $11 = 4^{\circ} \pm 0.66$		
Long chain	21	10.37 ± 1.47	11.00 ± 1.92	$11.70 \pm 0.03$	11.34 ± 0.00		
C17:0	7	$5.03^{b} + 0.33$	$5.65^{ab} \pm 0.09$	$6.27^{a} \pm 0.09$	$6.67^{a} + 0.43$		
617.0	21	$5.97^{a} + 0.01$	$5.78^{a} + 0.15$	$6.24^{a} + 0.30$	$6.24^{a} + 0.24$		
C17:1	7	$1.86^{\circ} \pm 0.06$	$2.05^{bc} \pm 0.02$	$2.64^{a_{*}} \pm 0.03$	$2.50^{ab} \pm 0.26$		
	21	$2.54^{a_{*}} \pm 0.04$	$2.59^{\rm a} \pm 0.42$	$2.33^{\rm a} \pm 0.09$	$2.39^{a} \pm 0.33$		
C18:0	7	$84.29^{a} \pm 0.17$	$87.90^{\mathrm{a}} \pm 1.82$	$82.65^{\mathrm{a}} \pm 1.19$	$95.81^{a} \pm 6.77$		
	21	$95.18^{b*} \pm 0.21$	$88.20^{\rm c} \pm 0.07$	$90.98^{c*} \pm 0.86$	$101.00^{\mathrm{a}} \pm 1.08$		
C20:0	7	$1.57^{ m a} \pm 0.30$	$1.90^{\rm a}\pm0.01$	$2.20^{a} \pm 0.03$	$2.04^{a} \pm 0.00$		
	21	$1.89^{\rm a} \pm 0.22$	$2.12^{a} \pm 0.28$	$2.02^{a}_{b} \pm 0.07$	$2.03^{\rm a}\pm0.05$		
C20:1	7	$0.83^{a} \pm 0.03$	$0.81^{a} \pm 0.00$	$0.63^{D} \pm 0.00$	$0.84^{a} \pm 0.04$		
	21	$0.79^{a} \pm 0.12$	$0.91^{a} \pm 0.11$	$0.95^{a*} \pm 0.02$	$0.96^{a} \pm 0.02$		
C20:2	/	$0.43^{\circ} \pm 0.04$	$0.31^{\circ} \pm 0.12$	$0.76^{a*} \pm 0.01$	$0.44^{\circ} \pm 0.00$		
C21-0	21	$0.63^{-5} \pm 0.02$	$0.68^{\circ} \pm 0.11^{\circ}$	$0.47^{\circ} \pm 0.05$ 1.40 <sup>a</sup> ± 0.02	$0.50^{\circ} \pm 0.08$		
021:0	21	$1.03 \pm 0.08$ $1.13^{a} + 0.14$	$1.13 \pm 0.02$ $1.27^{a} \pm 0.25$	$1.49 \pm 0.02$ $1.39^{a} \pm 0.04$	$1.41 \pm 0.00$ $1.42^{a} \pm 0.01$		
C22:6	7	$0.40^{b} \pm 0.14$	$5.60^{a*} \pm 0.23$	$1.39^{b} \pm 0.04^{c}$	$0.58^{b} + 0.29$		
022.0	21	$1.05^{a} \pm 0.16$	$1.47^{a} + 0.45$	$1.68^{a} \pm 0.41$	$1.59^{a*} \pm 0.03$		
C23:0	7	$1.42^{\circ} \pm 0.06$	$1.63^{\rm b} \pm 0.02$	$1.91^{a} \pm 0.02$	$1.85^{a} \pm 0.00$		
	21	$1.75^{a_{*}} \pm 0.04$	$2.58^{\rm a} \pm 0.45$	$1.85^{ m a} \pm 0.05$	$1.91^{a_{*}} \pm 0.01$		
C24:0	7	$0.22^{\mathrm{b}} \pm 0.01$	$0.28^{\rm b} \pm 0.04$	$0.46^{\mathrm{a}} \pm 0.00$	$0.31^{\mathrm{b}}\pm0.01$		
	21	$0.31^{a} \pm 0.15$	$0.31^{a} \pm 0.03$	$0.33^{\rm a} \pm 0.05$	$0.32^{a} \pm 0.07$		
C18:1n9t + C18:1ω9c	7	$191.08^{b} \pm 13.93$	$212.62^{ab} \pm 1.70$	$207.60^{ab} \pm 2.99$	$227.00^{a} \pm 0.00$		
	21	$228.22^{\text{b}} \pm 10.64$	$264.64^{a*} \pm 1.71$	$220.60^{\text{b}} \pm 12.33$	$228.59^{b} \pm 1.87$		
C18:2t	7	$0.93^{\circ} \pm 0.05$	$1.02^{bc} \pm 0.01$	$1.22^{a_{*}} \pm 0.01$	$1.07^{\text{D}} \pm 0.00$		
	21	$1.04^{a} \pm 0.15$	$1.15^{a} \pm 0.18$	$1.07^{a} \pm 0.03$	$1.03^{a} \pm 0.04$		
C18:2c	/	$15.78^{\circ} \pm 0.29$	$16.54^{\circ} \pm 0.27$	$17.52^{\circ} \pm 0.46$	$1/.49^{a} \pm 0.6/$		
C19-2-06	21	$18.21^{\circ} \pm 0.13^{\circ}$	$20.35^{-5} \pm 0.46$	$18.55^{\circ} \pm 0.48^{\circ}$	$18.01^{\circ} \pm 0.09$		
C18:300	21	$0.29 \pm 0.00$ $0.24^{a} \pm 0.02$	$0.37 \pm 0.02$ $0.20^{a} \pm 0.12$	$0.04^{-1} \pm 0.00^{-1}$	$0.40 \pm 0.04$ 0.25 <sup>a</sup> ± 0.02		
C20:3@6	7	$0.34^{\circ} \pm 0.03^{\circ}$	$0.35 \pm 0.12$ $0.85^{bc} \pm 0.03$	$1.16^{a*} + 0.01$	$0.33 \pm 0.02$ $0.94^{b} \pm 0.00$		
020.300	21	$1.04^{a} + 0.04$	$0.97^{a} \pm 0.11$	$0.96^{a} + 0.00$	$0.94^{a} \pm 0.00$ $0.94^{a} \pm 0.01$		
C20:3@3	7	$0.06^{\circ} \pm 0.00$	$0.08^{bc} \pm 0.00$	$0.22^{a_{*}} \pm 0.00$	$0.08^{b} \pm 0.00$		
	21	$0.26^{\rm a} \pm 0.20$	$0.13^{\mathrm{a}} \pm 0.03$	$0.10^{\mathrm{a}} \pm 0.01$	$0.09^{\rm a} \pm 0.00$		
C20:4w6	7	$0.10^{ m c} \pm 0.06$	$0.08^{\rm c}\pm0.00$	$0.25^{\rm b} \pm 0.00$	$0.57^{a_{\star}} \pm 0.01$		
	21	$0.15^{\mathrm{b}}\pm0.02$	$0.31^{a_{*}} \pm 0.03$	$0.23^{ab}\pm0.03$	$0.28^{ab}\pm0.03$		
C20:5ω3	7	$0.13^{\mathrm{a}} \pm 0.00$	$0.16^{a} \pm 0.02$	$0.29^{\rm a} \pm 0.00$	$0.51^{\mathrm{a}} \pm 0.48$		
	21	$0.18^{a} \pm 0.08$	$0.20^{a} \pm 0.01$	$0.21^{a} \pm 0.04$	$0.22^{a} \pm 0.03$		
C22:1ω9	7	$0.36^{a} \pm 0.03$	$0.18^{a} \pm 0.03$	$0.34^{a*} \pm 0.00$	$0.43^{a} \pm 0.36$		
624.4 0	21	$0.28^{a} \pm 0.00$	$0.24^{ab} \pm 0.03$	$0.19^{\text{b}} \pm 0.01$	$0.19^{\text{b}} \pm 0.00$		
024:109	/	$0.11^{\circ} \pm 0.00$	$0.15^{\circ} \pm 0.01$	$0.2/^{a*} \pm 0.00$	$0.31^{\circ} \pm 0.18$		
SCEA <sup>a</sup>	21	$0.26^{\circ} \pm 0.05$ 0.263* $\pm 0.26$	$0.20^{\circ} \pm 0.02^{\circ}$	$0.1/2 \pm 0.02$ 1 12 <sup>b</sup> * $\pm$ 0.01	$0.18^{-} \pm 0.02$ 1 20 <sup>b</sup> * ± 0.21		
JULA	21	$0.23^{a} + 0.05$	$1.42 \pm 0.30$ 0.13 <sup>a</sup> + 0.04	$0.12^{a} \pm 0.01$	$1.30 \pm 0.31$ $0.14^{a} \pm 0.00$		
	41	0.20 ± 0.00	0.10 ± 0.04	0.12 ± 0.00	0.14 ± 0.00		

#### Table 3 (Contd.)

		Creamy cheeses				
Fatty acid (mg $g^{-1}$ )	Days	CC	CLA	CBB	CLB	
MCFA <sup>a</sup>	7	$121.83^{\circ} \pm 2.51$	$136.46^{b} \pm 0.13$	$137.51^{\rm b} \pm 1.98$	$166.78^{a} \pm 4.40$	
	21	$138.06^{\mathrm{b}} \pm 0.93$	$134.22^{b} \pm 4.39$	$153.78^{ab} \pm 6.28$	$155.25^{a} \pm 0.04$	
LCFA <sup>a</sup>	7	$471.28^{\mathrm{b}} \pm 13.85$	$531.09^{ab} \pm 4.37$	$525.11^{\mathrm{ab}} \pm 6.86$	593.22 <sup>a*</sup> ± 31.9	
	21	$539.79^{b} \pm 8.28$	$592.54^{a*} \pm 2.34$	$564.69^{\mathrm{ab}} \pm 17.16$	$585.91^{a} \pm 2.32$	
$SFA^b$	7	$369.30^{\circ} \pm 1.59$	$406.41^{b} \pm 1.91$	$403.58^{\mathrm{b}} \pm 5.82$	$464.21^{a} \pm 10.5$	
	21	$399.27^{b*} \pm 3.64$	$413.54^{\mathrm{b}} \pm 0.78$	$445.13^{a*} \pm 10.56$	$460.80^{a} \pm 1.24$	
MUFA <sup>b</sup>	7	$209.83^{\mathrm{a}} \pm 14.49$	$232.88^{\rm a} \pm 1.54$	$231.06^{a} \pm 3.33$	$267.65^{a} \pm 25.4$	
	21	$246.85^{\mathrm{b}} \pm 9.43$	$284.68^{a*} \pm 1.39$	$243.41^{\mathrm{b}} \pm 12.72$	$250.24^{b} \pm 1.12$	
$PUFA^b$	7	$29.84^{\mathrm{a}} \pm 1.43$	$29.84^{\mathrm{a}} \pm 1.43$	$29.36^{a} \pm 0.29$	$29.82^{a} \pm 0.26$	
	21	$32.59^{a} \pm 1.12$	$32.59^{a} \pm 1.12$	$30.26^{a} \pm 0.21$	$30.39^{a} \pm 0.03$	
C18:2c9t11 (CLA) <sup>c</sup>	7	$3.05^{\rm c} \pm 0.07$	$5.30^{ m b} \pm 0.26$	$5.88^{\mathrm{b}}\pm0.08$	$7.69^{a} \pm 0.12$	
	21	$5.20^{c_{*}} \pm 0.00$	$6.89^{ab} \pm 0.12$	$6.59^{\mathrm{b}}\pm0.33$	$7.35^{a} \pm 0.08$	

<sup>*a*</sup> SCFA (C4:0–C9:0), short-chain fatty acids; MCFA (C10:0–C15:1), medium-chain fatty acids; and LCFA (C16:0–C24:0), long-chain fatty acids. <sup>*b*</sup> SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids. <sup>*c*</sup> CLA = conjugated linoleic acid. <sup>*a*-c</sup>Means  $\pm$  standard deviation with different letters in the same line differ by Tukey's test ( $p \le 0.05$ ). \*Means  $\pm$  standard deviation in the same column differ from each other by Student's *t*-test. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter starter and 8% inulin.

increased CLA content in synbiotic cheese with added FOS, inulin, *B. lactis* and *L. casei* compared to cheeses with only added probiotics.

The use of goat milk for the production of synbiotic creamy cheeses may be more advantageous than the use of bovine milk due to the greater availability of linoleic acid, which may result in higher CLA contents.<sup>82</sup> Microbial CLA production depends on the presence of free linoleic acid in the medium, allowing the formation of 10-hydroxy-12-octadecanoic acid and the isomerization into CLA.<sup>81</sup> The presence and formation of CLA in cheese are desirable because this fatty acid comprises a mixture of geometric and positional isomers of linoleic acid with important biological properties, including anticarcinogenic and immunomodulatory effects that reduce atherosclerosis, obesity and diabetes.<sup>83</sup>

**Proteolysis and free amino acids.** With respect to the EPI, the highest values ( $p \le 0.05$ ) were observed for CLA and CLB on the 21<sup>st</sup> day of storage compared to CC, with no EPI differences (p > 0.05) between CBB and CC (Table 4).

Higher DPI (secondary proteolysis) values ( $p \le 0.05$ ) were observed in CLB on the 21<sup>st</sup> day of storage, suggesting that the addition of probiotic bacteria resulted in increased proteolysis in the cheeses, with a potential for the greatest release of peptides and free amino acids.<sup>69</sup> The increased DPI in CLB may be associated with the joint action of probiotic cultures, which have a complex proteolytic system in which enzymes act primarily to degrade intermediate-size peptides produced from casein as a result of chymosin and plasmin activity.<sup>84</sup>

Among the 17 amino acids detected in assessed creamy goat cheeses, 8 were essential amino acids (isoleucine, leucine, phenylalanine, lysine, tryptophan, valine, tyrosine and histidine) (Table 4) that stood out in the amino acid profiles of these products. PCA was used to investigate the ability of probiotics and inulin to release amino acids based on the major components that define cheeses evaluated on the  $7^{\rm th}$  and  $21^{\rm st}$  days of cold storage (Fig. 3).

The two principal components PC1 and PC2 presented variances of 46% and 20%, respectively. The CC samples on the 7<sup>th</sup> and 21<sup>st</sup> days of storage were characterized by the presence of large amounts of tryptophan, threonine, methionine and histidine. Fig. 3 shows that the co-culture of probiotics and the presence of inulin (CLB) exerted a greater influence on the release of amino acids on the 21<sup>st</sup> day of storage. This result confirms the higher activity of probiotic bacteria when added to cheeses in co-cultures. Bezerra *et al.*<sup>16</sup> studied probiotic curd cheese and observed a similar behaviour, with a high release of amino acids and an apparent effect on the presence of glycine and lysine.

However, the CBB and CLA cheeses had less influence on the release of amino acids. The production of free amino acids contributes to the flavour characteristics of cheeses and generates precursors of catabolic reactions that produce keto acids, ammonia, amines, aldehydes, acids and alcohols that contribute to the characteristic flavour and aroma of cheeses.<sup>85</sup>

## Sensory analysis

The mean values for the sensory analysis of creamy goat cheeses containing or not containing inulin and probiotic bacteria (*L. acidophilus* LA-05 and/or *B. lactis* BB-12) on the 7<sup>th</sup> and 21<sup>st</sup> days of cold storage are shown in Table 5.

The scores assigned to the attributes of appearance, colour, aroma, texture, flavour and overall assessment ranged from 5.68 (neither liked nor disliked) to 8.06 (liked very much), and the lowest and highest values were obtained for flavour. In this sense, the four creamy goat cheeses were considered well-accepted because their scores were outside the rejection region (from 1 to 4 points).<sup>38</sup>

Table 4	Mean values ± standard deviation of free amino acids and proteolysis of goat creamy cheese with or without addition of inulin and/or pro-
biotics o	the 7 <sup>th</sup> and 21 <sup>st</sup> days of storage

		Creamy cheeses			
Free amino acids (mg per 100g)	Days	CC	CLA	CBB	CLB
Aspartic acid	7	$3.47^{d_{*}} \pm 0.01$	$4.83^{b_{*}} \pm 0.01$	$4.69^{c*} \pm 0.01$	$5.12^{a_{*}} \pm 0.01$
Ĩ	21	$3.35^{\mathrm{b}} \pm 0.00$	$3.51^{\mathrm{b}} \pm 0.00$	$2.95^{\rm c} \pm 0.07$	$4.18^{a} \pm 0.02$
Glutamic acid	7	$3.17^{ m c} \pm 0.01$	$5.04^{b_{*}} \pm 0.01$	$1.65^{d} \pm 0.02$	$10.69 \pm 0.01^{a}$ *
	21	$3.62^{b_{*}} \pm 0.00$	$1.79^{ m c} \pm 0.01$	$1.24^{\rm d} \pm 0.00$	$9.58 \pm 0.02^{a}$
Serine	7	$9.62^{a_{*}} \pm 0.01$	$3.79^{b_{*}} \pm 0.02$	$1.02^{\rm c} \pm 0.01$	$3.85^{b} \pm 0.01$
	21	$3.61^{ m b} \pm 0.01$	$2.01^{\rm c} \pm 0.03$	$2.07^{c_{*}} \pm 0.02$	$5.25^{a_{*}} \pm 0.01$
Glycine	7	$2.03^{c_{*}} \pm 0.01$	$3.94^{a} \pm 0.03$	$2.35^{b*} \pm 0.01$	$2.36^{b} \pm 0.01$
2	21	$0.70^{ m d} \pm 0.01$	$4.00^{\mathrm{a}}\pm0.01$	$1.38^{\rm c} \pm 0.01$	$3.75^{b*} \pm 0.01$
Histidine	7	$14.40^{\mathrm{b}}\pm0.00$	$14.12^{\mathrm{a}} \pm 0.01$	$11.77^{\rm d} \pm 0.03$	$13.10^{c_{*}} \pm 0.07$
	21	$14.35^{\mathrm{a}} \pm 0.03$	$14.03^{ m b} \pm 0.07$	$12.07 \pm 0.07^{c_{*}}$	$11.21^{d} \pm 0.00$
Arginine	7	$41.98^{a_{*}} \pm 0.01$	$38.85^{b*} \pm 0.01$	$10.81^{\rm d} \pm 0.01$	$33.96^{\ c} \pm 0.01$
0	21	$19.14^{\rm d} \pm 0.01$	$34.45^{\circ} \pm 0.01$	$35.53^{b*} \pm 0.01$	$53.58^{a_{*}} \pm 0.01$
Threonine	7	$3.65^{d} \pm 0.01$	$4.08^{ m c} \pm 0.01$	$23.25^{a*} \pm 0.01$	$8.84^{\mathrm{b}}\pm0.01$
	21	$30.79^{a_{*}} \pm 0.03$	$5.02^{c_{*}} \pm 0.01$	$5.20^{\circ} \pm 0.21$	$11.98^{b} \pm 0.01$
Alanine	7	$3.54^{\rm b} \pm 0.03$	$2.76^{\rm c} \pm 0.01$	$1.05^{\rm d}\pm0.01$	$6.42^{a} \pm 0.06$
	21	$4.90^{c*} \pm 0.03$	$5.80^{b*} \pm 0.01$	$5.82^{b*} \pm 0.03$	$16.62^{a_{*}} \pm 0.06$
Proline	7	$16.75^{\rm b} \pm 0.03$	$9.88^{ m c} \pm 0.01$	$8.78^{\rm d} \pm 0.01$	$24.47^{a} \pm 0.05$
	21	$15.40^{ m b} \pm 0.01$	$9.35^{\circ} \pm 0.10$	$9.65^{c_{*}} \pm 0.01$	$28.70^{a} \pm 0.01$
Tyrosine	7	$6.08^{ m a} \pm 0.01$	$5.14^{\mathrm{b}} \pm 0.02$	$4.16^{ m c} \pm 0.05$	$5.90^{a} \pm 0.10$
5	21	$6.01^{\mathrm{b}}\pm0.01$	$5.54^{ m c} \pm 0.12$	$5.67^{c*} \pm 0.08$	$9.63^{a_{*}} \pm 0.02$
Valine	7	$3.97^{\rm c} \pm 0.01$	$5.33^{ m b} \pm 0.01$	$2.88^{\rm d} \pm 0.10$	$9.50^{a} \pm 0.02$
	21	$4.33^{c*} \pm 0.01$	$5.55^{\mathrm{b}}\pm0.41$	$5.84^{b*} \pm 0.01$	$16.89^{a_{*}} \pm 0.4$
Methionine	7	$1.52^{ m c} \pm 0.50$	$3.56^{\mathrm{a}} \pm 0.01$	$1.86^{\rm c} \pm 0.01$	$2.49^{ m b} \pm 0.01$
	21	$7.23^{a_{*}} \pm 0.00$	$3.07^{\rm b} \pm 1.30$	$3.39^{b*} \pm 0.55$	$3.32^{b*} \pm 0.00$
Isoleucine	7	$1.65^{ m c} \pm 0.01$	$2.09^{\rm b} \pm 1.30$	$1.25^{\rm c} \pm 0.10$	$4.82^{a} \pm 0.30$
	21	$5.24^{b*} \pm 0.01$	$3.30^{c*} \pm 0.01$	$1.84^{\rm d} \pm 0.01$	$8.71^{a_{*}} \pm 0.01$
Leucine	7	$7.44^{b} \pm 0.30$	$7.77^{b*} \pm 0.01$	$6.47^{c*} \pm 0.10$	$12.79^{a} \pm 0.01$
	21	$9.09^{b*} \pm 0.01$	$6.45^{\rm c} \pm 0.01$	$5.66^{\rm d} \pm 0.00$	$37.25^{a_{*}} \pm 0.01$
Phenylalanine	7	$9.31^{a} \pm 0.01$	$2.68^{\rm d} \pm 0.01$	$5.12^{c} \pm 0.00$	$8.29^{ m b} \pm 0.00$
5	21	$11.34^{b*} \pm 0.01$	$5.16^{d} \pm 0.01$	$7.53^{c*} \pm 0.00$	$23.02^{a_{*}} \pm 0.01$
Lysine	7	$6.99^{d} \pm 0.02$	$11.88^{\rm c} \pm 0.10$	$35.62^{a_{*}} \pm 0.01$	$17.17^{b} \pm 0.01$
<b>J</b>	21	$7.16^{\rm d} \pm 0.22$	$33.95^{b*} \pm 0.11$	$12.47^{\rm c} \pm 0.01$	$37.04^{a_{*}} \pm 0.01$
Tryptophan	7	$21.89^{a} \pm 0.11$	$11.72^{\rm b} \pm 0.01$	$10.92^{\rm c} \pm 0.22$	$11.39^{b} \pm 0.03$
	21	$22.20^{a*} \pm 0.01$	$11.59^{\rm c} \pm 0.01$	$11.37^{c*} \pm 0.01$	$14.52^{b*} \pm 0.01$
Proteolysis					
$EPI^{a}(\%)$	7	$15.36^{\mathrm{b}} \pm 0.11$	$16.00^{ m ab} \pm 0.36$	$14.96^{b} \pm 0.23$	$16.39^{a} \pm 0.46$
	21	$15.33^{b} \pm 0.25$	$16.73^{a} \pm 0.20$	$15.30^{ m b} \pm 0.34$	$17.21^{a_{*}} \pm 0.11$
$\text{DPI}^{b}(\%)$	7	$16.25^{a} \pm 0.42$	$14.00^{b} \pm 0.62$	$15.66^{a_{*}} \pm 0.60$	$15.32^{a} \pm 0.80$
	21	$16.58^b\pm0.01$	$14.33^d\pm0.01$	$12.33^{c} \pm 0.12$	$17.09^{a_{*}} \pm 0.01$

<sup>*a*</sup> EPI = proteolysis extent. <sup>*b*</sup> DPI = proteolysis depth. <sup>*a*-d</sup>Means  $\pm$  standard deviation with different letters in the same line differ by Tukey's test ( $p \leq 0.05$ ). Culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8%; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin. \* Means  $\pm$  standard deviation in the same column differ from each other by Student's *t*-test.

Regarding appearance, the highest and lowest scores ( $p \le 0.05$ ) were obtained for CLB and CC, respectively, on the 21<sup>st</sup> day of storage, and no differences were observed (p > 0.05) among CC, CLA and CBB in the same storage time interval. On the 7<sup>th</sup> day of storage, no differences (p > 0.05) were observed in the appearance of the different types of cheeses evaluated, except for CLA.

The colour, aroma and texture of CLA, CBB and CLB did not differ (p > 0.05) compared to CC on the 21<sup>st</sup> day of storage. The highest flavour scores were observed for CLA and CBB compared to CC and CLB on the 7<sup>th</sup> and 21<sup>st</sup> days of storage. Regarding CLA and CBB, the addition of *L. acidophilus* and *B. lactis* may have improved the taste due to the reduced acidification of the product compared to CLB, as observed in Fig. 2. The CLB cheese with added probiotic co-culture did not differ (p < 0.05) in relation to the flavour of CC. Different species of *Lactobacillus* and *Bifidobacterium* in co-culture may alter cheese flavour by accentuating the bitter, acetic and acidic flavours,<sup>86</sup> which may have occurred in CLB that obtained lower flavour scores than CBB and CLA.

With regard to the overall assessment, all of the creamy cheeses evaluated had similar scores (p > 0.05) on the  $21^{st}$  day of storage, with intermediate scores located between hedonic terms "liked slightly" and "liked moderately" (Table 5).

The ideal condition for the addition of probiotic cultures and prebiotic ingredients in cheese is that they do not interfere with the sensory characteristics and do not decrease consumer acceptance of the product. In this study, the addition of probiotics and prebiotics into goat creamy cheeses resulted in a similar or better



**Fig. 3** PCA graphics of the free amino acid profile of goat creamy cheese with or without the addition of inulin and/or probiotics on the 7th and 21st days of storage. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *lactis* and *S* inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin.

Table 5	Mean values $\pm$ standard deviation of the acceptance and purchase intent tests of goat crean	my cheese with an	d without additio	n of inulin
and/or p	obiotics on the 7 <sup>th</sup> and 21 <sup>st</sup> days of storage			

	Days	Creamy cheeses				
Sensory analysis		CC	CLA	CBB	CLB	
Appearance	7	$7.36^{a*} \pm 0.97$	$6.85^{b} \pm 1.02$	$7.00^{ m ab} \pm 1.08$	$7.18^{ab} \pm 0.96$	
	21	$6.28^{ m b} \pm 1.58$	$6.73^{ab} \pm 1.38$	$6.76^{ab} \pm 1.22$	$7.11^{a} \pm 1.29$	
Color	7	$7.25^{\mathrm{a}} \pm 1.51$	$7.06^{\rm a} \pm 0.93$	$7.06^{a} \pm 0.73$	$6.90^{\rm a} \pm 0.83$	
	21	$6.73^{a} \pm 1.43$	$6.60^{ m a} \pm 1.27$	$7.06^{a} \pm 1.30$	$6.71^{a} \pm 1.23$	
Aroma	7	$7.26^{ab} \pm 1.08$	$6.95^{bc} \pm 0.59$	$7.50^{a*} \pm 0.96$	$6.83^{c} \pm 0.90$	
	21	$6.61^{a} \pm 1.29$	$6.75^{\mathrm{a}} \pm 1.17$	$6.73^{a} \pm 1.05$	$6.60^{ m a} \pm 0.61$	
Flavor	7	$7.10^{b*} \pm 0.93$	$8.06^{a_{*}} \pm 0.91$	$7.96^{a_{*}} \pm 0.66$	$6.73^{b_{*}} \pm 1.42$	
	21	$6.01^{ m b} \pm 1.51$	$6.73^{a} \pm 0.98$	$7.05^{a} \pm 1.32$	$5.68^{b} \pm 1.66$	
Texture	7	$7.35^{a_{\star}} \pm 0.97$	$7.30^{a*} \pm 0.97$	$7.50^{a_{*}} \pm 0.87$	$7.18^{a} \pm 1.28$	
	21	$6.73^{a} \pm 1.00$	$6.78^{ m a} \pm 1.01$	$6.71^{a} \pm 1.04$	$6.45^{a} \pm 1.11$	
Overall assessment	7	$7.18^{b*} \pm 1.12$	$7.73^{a_{*}} \pm 0.66$	$7.31^{ab} \pm 1.21$	$7.18^{b} \pm 1.37$	
	21	$6.70^{ m a} \pm 0.99$	$6.83^{a} \pm 1.04$	$6.85^{a} \pm 1.13$	$6.68^{a} \pm 1.04$	
Purchase intent	7	$4.05^{a_{\star}} \pm 0.99$	$4.01^{a_{*}} \pm 0.83$	$3.90^{a_{*}} \pm 1.08$	$3.68^{a_{*}} \pm 0.85$	
	21	$2.75^{bc} \pm 0.72$	$3.26^{a}\pm0.44$	$3.05^{ab} \pm 1.03$	$2.58^{c_{*}} \pm 1.06$	

<sup>a-c</sup>Means ± standard deviation with different letters in the same line differ by Tukey's test ( $p \le 0.05$ ). \*Means ± standard deviation in the same column differ from each other by Student's *t*-test. CC – control goat creamy cheese added with conventional starter culture, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; CLA – symbiotic goat creamy cheese added with starter culture, probiotic *Lactobacillus acidophilus* and 8% inulin; CBB – symbiotic goat creamy cheese added with starter culture, probiotic *Bifidobacterium animalis* subsp. *lactis* and 8% inulin; CLB – symbiotic goat creamy cheese added with starter cultures and 8% inulin.

appearance, colour, aroma, texture and flavour compared to conventional cheese, confirming the possibility of using such technologies without decreasing consumer acceptance.

Regarding the purchase intention, average scores of 3–5 points were considered as the acceptance interval for the formulations.<sup>38</sup> On the 7<sup>th</sup> day of storage, all of the types of creamy cheeses evaluated received scores within the acceptance interval, and the highest scores (in seat 4) were observed for CC and CLA, corresponding to the hedonic term "possibly would buy" (Table 5). After 21 days of storage, only CC and CLB were outside the acceptance interval regarding purchase intention but were not different (p > 0.05) among them. This result may be related to the fermentation activity of the starter culture and/or probiotic co-culture, resulting in increased acidity and reduced pH as observed in this study, influencing negatively the flavour of products.

The CC and CLB obtained the lowest scores for purchase intention and also for flavour, confirming that flavour is probably the most important attribute in the choice of a certain food, followed by the health benefits provided by the food product. In this sense, when functional ingredients are added to a particular food, consumers need to be made aware of the health benefits to perceive the functional food as healthier than conventional foods<sup>87</sup> and thus generate a positive influence on the purchase intention of these products.

# Conclusions

Synbiotic creamy goat cheese with added inulin, L. acidophilus LA-05 and/or B. lactis BB-12 showed improved technological, physicochemical and sensory characteristics compared to creamy goat cheese prepared with only a starter culture composed of Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris. The probiotics counts and fructan quantification showed that creamy goat cheese is a good carrier of probiotic cultures and inulin, and amounts of these components sufficient to promote health benefits to consumers were detected. Regarding the technological aspects of texture, cheeses with added inulin and probiotic cultures were less firm and had a better consistency. In relation to the physicochemical aspects, changes in fatty acid profile stand out, showing an increase in the amount of CLA in cheeses containing inulin and probiotic cultures. Lactic acid was the main organic acid identified in the evaluated cheeses, and the amounts of this organic acid increased over the storage period, resulting probably in increased acidity and reduced pH. In relation to the amino acid profile, the availability of a variety of essential amino acids was observed in the different types of creamy goat cheeses, and the greatest release of amino acids occurred in CLB. Sensory analysis showed that the cheeses were well-accepted by consumers regarding the attributes of appearance, colour, flavour, texture and overall assessment. Thus, creamy goat cheese was a good carrier of inulin, as well as of probiotic cultures of L. acidophilus and B. lactis, providing possible beneficial effects to consumer health related to the consumption of products with synbiotic characteristics.

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