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Effect of temperature and concentration of βeta-galactosidase on the composition of reduced lactose pasteurized goat milk

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Abstract

This study evaluated the influence of temperature and β-galactosidase concentration on lactose hydrolysis and on the composition of pasteurized goat milk. Goat milk was pasteurized at 75 °C/15 s and cooled to 10 °C and 30 °C, received 0 (control); 0.04%; 0.07%; 0.20% (v/v) β-galactosidase and was incubated for 5 h, followed by enzymatic inactivation at 85 °C. The hydrolysis degree, pH and acidity were evaluated hourly. Physico-chemical parameters were determined after hydrolysis. The maximum hydrolysis degree (100%) has been reached in 4 h when using 0.07% and 0.20% lactase concentrations at 30 °C; however, the minimum hydrolysis percentage of 70% has been reached at 10 °C for all lactase concentrations tested since 1 h of incubation. The degree of hydrolysis and the total acidity of pasteurized goat milk increased with temperature. Low lactase concentrations resulted in an increase in protein levels, total casein, density, total and defatted dry extract. Therefore, combination of low lactase levels and hydrolysis at 10 °C promoted positive changes in lactose-free pasteurized goat milk. This study was the first reporting the changes resulting from enzymatic hydrolysis in the composition of pasteurized goat milk.

Keywords: cryoscopic index; lactose intolerance; enzymatic hydrolysis; caprine milk.

Practical Application: Quality lactose-free goat milk using low temperature and β-galactosidase concentration.

1 Introduction

Goat milk production and commercialization is traditional in South America; however, it has been affected by continuous regional economic fluctuations. The establishment of commercial exploitations and the implementation of programs to improve this activity have driven milk production and adequate technology that is currently available makes it possible to continue improving the conditions of traditional goat production systems (Ramón et al., 2018). Moreover, consumers expect high food quality, sensory attractiveness and appropriate nutritional value of food, associated to health-promoting properties, which are also present in goat milk products (Barlowska et al., 2018; Mituniewicz-Malek et al., 2019).

The prevalence of diseases related to the consumption of certain foods has increased considerably in recent years, with lactose intolerance and bovine milk protein allergy being the most frequent. Excluding this product from the diet is the main recommendation of health professionals. Goat milk is recognized for its nutritional and hypoallergenic properties, being indicated in the diet for children allergic to bovine milk (Pradeep Prasanna & Charalampopoulos, 2019; Beltrán et al., 2017). Research related to goat milk has also indicated that its proteins as well as the peptides produced from them have important biological activity such as antimicrobial, immunomodulator, antioxidant, hypocholesterolemic and antihypertensive activities (Medeiros et al., 2018; Mal et al., 2018). It has better digestibility, greater bioavailability of iron and magnesium and higher calcium and copper content than bovine milk (Lucatto et al., 2020; Fangmeier et al., 2019). The composition of long-chain fatty acids in its fraction of monounsaturated and polyunsaturated fatty acids (ω-6 and ω-3 fatty acids, eicosapentaenoic acid- EPA and docosahexaenoic acid- DHA, and medium-chain triglycerides) present in goat milk which are considered beneficial to human physiology surpasses bovine milk, (Hodgkinson et al., 2018); and is also rich in niacin, thiamine, riboflavin, and pantothenate (Ranadheera et al., 2019).

Caprine (goat) milk also possesses potential for successful delivery of probiotics, and despite its less appealing flavor in some products, the use of goat milk as a probiotic carrier has rapidly increased over the last decade (Ranadheera et al., 2019). Dairy products with goat milk have been developed, as for instance yogurt (Hadjimbei et al., 2020), 'dulce de leche' (Chaves et al., 2018), cheese (Shabbir et al., 2019; Özturk & Akin, 2018), cream cheese (Fangmeier et al., 2019) and fermented reconstituted whey (Santos et al., 2019).

Despite the advantages, goat milk is not indicated in the diet of lactose intolerant individuals, with the main alternative for this differentiated group being the consumption of milk and derivatives which are free of and/or low in sugar. Lactose intolerance is due to the lack or low content of β-galactosidase (lactase) present in the body capable of hydrolyzing lactose after ingestion of milk or milk products (Nardi et al., 2017). According to Zolnere &

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Ciprovica (2017), enzymatic hydrolysis has been referred to as the most used method by the food industry to obtain bovine milk free of and/or low in lactose. This method consists in the hydrolysis of the lactose glycosidic bond through β-galactosidase, resulting in glucose and galactose, two monosaccharides which are easily absorbed by the body.

However, besides transforming lactose into simple monosaccharides, there may be other changes in the composition of milk, as reported by Trevisan (2008), who observed a slight increase in protein content, relative density, total and defatted dry extract of hydrolyzed semi-skimmed bovine milk with a concentration of 0.09% lactase incubating at 36 °C using the ultrasonic method of analysis. Therefore, this is the first research to investigate such changes resulting from enzymatic hydrolysis in the composition of pasteurized goat milk. In addition, most available studies on lactose enzymatic hydrolysis of bovine milk employ temperatures between 30 and 40 °C, which is ideal for both lactase action and the development of lactic and pathogenic bacteria (El-Kader et al., 2012; Bosso et al., 2016), with it therefore being essential to evaluate the effect of lower temperatures, including maximum refrigeration on milk production (Nakagawa et al., 2007; Antunes et al., 2014). Therefore, the objective of this work was to evaluate the influence of temperature and concentration of β-galactosidase (lactase) on lactose hydrolysis and the resulting changes in the composition of pasteurized goat milk.

2 Materials and methods

Fresh goat milk was purchased from the goat sector of the Academic Unit Specialized in Agricultural Sciences of the Federal University of Rio Grande do Norte, Campus of Macaíba-RN. It was then transported to the same unit's Dairy Processing Laboratory, where it was homogenized using polypropylene spatula, filtered through nylon screen (18 × 29 × 16 cm), and pasteurized using a water bath and controlling the temperature increase during 15 min by manual thermometer (Incoterm, São Paulo, Brasil), until reach 75 °C/15 s, then cooled to two different temperatures of 10 and 30 °C and divided into 400 mL portions which were added from 0 (control); 0.04%; 0.07%; and 0.20% (v/v) β-galactosidase from *Kluyveromyces lactis* yeast with lactase activity of 50.000 ONPGU/g (Prozyn, São Paulo, Brazil).

The lactase-added portions of goat milk were stored in an incubator (Tecnal, São Paulo, Brasil) for five hours maximum at the same temperatures (10 and 30 °C). Then, representative samples of 400 mL were removed at one-hour intervals, heat-treated at 85 °C for enzymatic inactivation and immediately cooled to 20 °C.

2.1 Physicochemical analysis

Samples were evaluated at one-hour intervals for a five-hour period for titratable acidity and pH (Association Official Analytical Chemists, 2000). The cryoscopy (°H) index was also determined using the digital electronic cryoscope (Model MK 540 Flex, Brazil) and transferring 2.5 mL samples directly to the instrument measurement cell. The degree of hydrolysis was calculated according to the methodology used by Moreira et al. (2009). The parameters of relative density, protein content, total casein, fat, total dry extract and defatted dry extract were evaluated after five hours of hydrolysis by the infrared radiation method using Bentley 2000 mid-infrared instrument (DairySpec FT, USA) (Sales et al., 2018). To this end, 40 mL of hydrolyzed goat milk was placed in a cuvette and introduced into the instrument. All analyzes were performed in triplicate.

Lactose was determined by high performance liquid chromatography (HPLC) after a hydrolysis period of five hours according to the method proposed by Burgner & Feinberg (1992), after extraction in aqueous medium with potassium ferrocyanide solution (Carrez I) and zinc sulfate (Carrez II). An Agilent Technologies (Infinity 1260, USA) liquid chromatograph with automatic sampler (20 µL injection), quaternary solvent system, column oven and an ELSD detector was used. The chromatographic conditions were: Zorbax Carbohydrate column (4.6 mm ID × 250mm) 5 µm; column temperature 30 °C; acetonitrile mobile phase: water (80:20) with flow rate of 1.1 mL/min; injection volume 20 µL; nebulization temperature: 50 °C; evaporation temperature: 80 °C.

2.2 Microbiological analyzes

All samples were submitted in triplicate to the determination of total coliforms and mesophilic bacteria, as recommended by the technical regulation for pasteurized goat milk (Brasil, 2000) and thermotolerant coliforms and *Salmonella sp*., required by the National Health Surveillance Agency for goat milk (Brasil, 2001). Analyzes were performed according to the methodology described by the American Public Health Association (2001).

2.3 Experimental design and statistical analysis

The experiments were performed in triplicate using a completely randomized design (DIC) with independent variables being time, temperature and lactase concentration (Appendix A - Table A1). Shapiro-Wilk test was used to evaluate normality of the results. Analysis of variance one-way ANOVA followed by comparison of means by the Tukey test at 5% significance level using the Statistica version 7.0 software program were applied.

3 Results and discussion

Thermotolerant coliforms at 45 °C, *Salmonella sp*. and mesophilic aerobic bacteria were not found in the processed milk samples, indicating efficiency in the goat milk pasteurization process employed in this study.

The degree of hydrolysis of the samples with addition of 0.04; 0.07 and 0.20% lactase differed significantly depending on the concentration of the added enzyme ($p < 0.05$) in the first hour of incubation at 30 ° C. The same behavior was observed in samples stored at 10 ° C for a longer period (4 h) (Figure 1; Appendix A - Table A2). These results are consistent with previous research by Horner et al. (2011) with pasteurized bovine milk, using other conditions of temperature, time and higher concentrations of lactase. The results obtained in this study, however, demonstrated greater efficiency of hydrolysis, since the use of lower concentrations of lactase are more cost effective for commercial production.

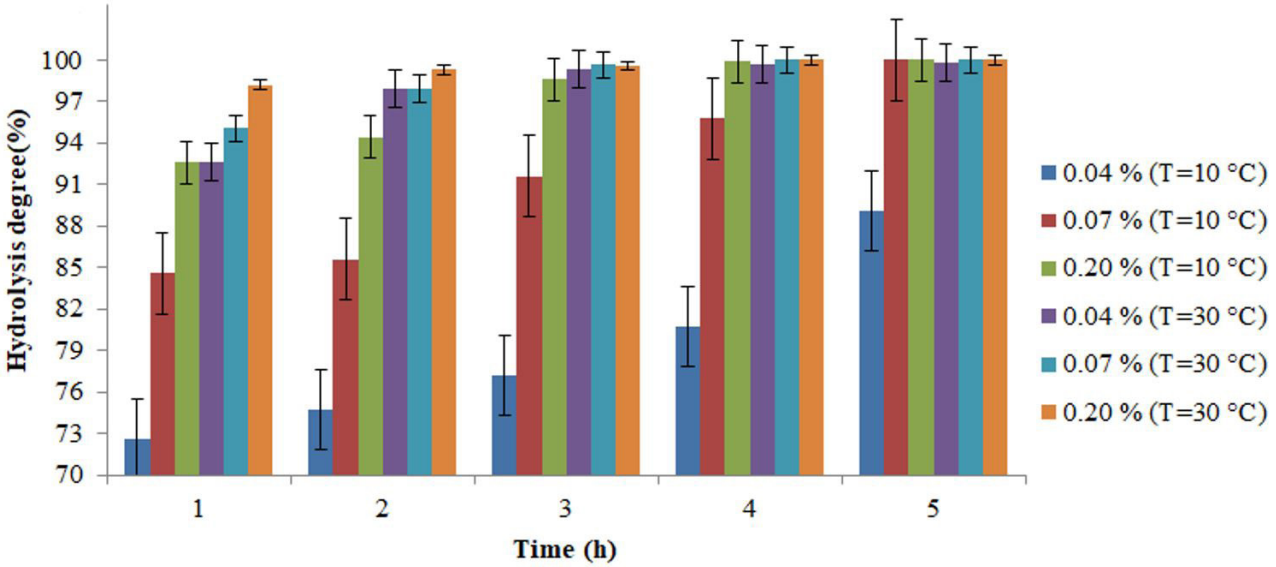

Figure 1. Hydrolysis degree (%) of pasteurized goat milk with different lactase concentrations (0.04%, 0.07% and 0.20% v/v) incubated at 10 °C and 30 °C during 5 h.

The hydrolysis percentages showed significant difference ($p < 0.05$) as a function of time and temperature of incubation, except for the results related to the three different lactase concentrations from 4 to 5h incubation at 30 °C and after 5h to 0.20% lactase-added sample incubated at 10 °C. The maximum hydrolysis degree (100%) was reached within 5h using 0.07% and 0.20% lactase at 10 °C, while the same percentages were obtained after 4h of incubation at 30 °C using the same enzyme concentrations. These results showed that although hydrolysis using higher lactase concentrations at 30 °C is higher in a shorter time interval, the hydrolysis percentage surpasses 70% using the temperature of 10 °C for all lactase concentrations tested at all incubation time intervals. Lactose reduction from 70 to 80% in dairy products is enough for most people who are lactose intolerant (Hourigan, 1984) and such range of lactose reduction may be considered as satisfactory (Rosolen et al., 2015).

Pasteurized goat milk without lactase (control) showed 0.17% lactic acid, fat content 3.8% w/w, relative density 1.034 g/mL at 15 °C, 3.9% w/w protein, 85.3% w/w total casein, 4.3% w/v lactose, defatted and total dry extract of 10.2 and 14.0% w/w, respectively, and cryoscopy of -0.585 °H. These results are within the standard established by current regulations, which recommend values ranging from 0.13% to 0.18% lactic acid; relative density ranging from 1.028 to 1.034 g / mL at 15 °C; at least 2.8% w/w protein; minimum lactose 4.3% w/v; minimum defatted dry extract of 8.2% w/w; and cryoscopy ranging from -0.550 to -0.585 °H (Brasil, 2000).

Although samples incubated at 30 °C achieved a higher degree of hydrolysis (100%) in a shorter period compared to those incubated at 10 °C, their total acidity was already higher than the control (0.17%), reaching values ranging from 0.19% to 0.21% lactic acid after 1 h and 5h ($p < 0.05$) (Table 1). Similarly, significant changes in pH ($p < 0.05$) were observed, the values of which were reduced from 6.6 to 6.1 as total acidity increased ($p < 0.05$) (Table 2). Thus, it was shown that the pH and acidity results showed a good correlation between themselves, differing significantly as a function of temperature ($p < 0.05$).

In view of the technological application, the pH and acidity values disagreeing with the standard indicate that the temperature employed (30 °C) for 5 h is inappropriate to obtain free of or low lactose goat milk for both direct consumption and for use in obtaining its products. In contrast, hydrolyzed goat milk at 10 °C maintained total acidity in the range of 0.18% lactic acid over the same time period, in line with legislation (Brasil, 2000) while pH varied from 6.7 to 6.4 normal values referenced in previous research with goat milk (Altinçekiç & Koyuncu, 2017). These results corroborate the findings of Antunes et al. (2014), who obtained 0.14% lactic acid content and pH 6.76 in skim bovine milk samples submitted to enzymatic hydrolysis using 0.4 mL/L lactase from *Kluyveromyces lactis* for 21 h at 10 ± 1 °C followed by the microfiltration process.

The different incubation temperatures and lactase concentrations used did not significantly affect ($p > 0.05$) the levels of fat, protein and density, while the total casein levels were significantly affected ($p < 0.05$) (Table 3). There was also an increase in, protein, total casein, relative density, total and defatted dry extract when compared to those obtained in the control sample (without lactase addition) ($p < 0.05$). These results may be associated to the lactose hydrolysis reaction with consequent increase in total milk solids, as already reported by Trevisan (2008), who observed a slight increase in protein content, relative density, total and defatted dry extract of hydrolyzed semi-skimmed bovine milk.

Lactose results (Table 3) did not differ significantly as a function of temperature; however, there was a significant difference as a function of enzyme concentration for samples added with 0.04% lactase compared with those with 0.07% and 0.20%.

Table 1. Total acidity values of pasteurized goat milk incubated at 10 °C and 30 °C with different lactase concentrations (0.04%, 0.07% and 0.20% v/v) during 5 h.

Time (h)	Lactase %			
	Temperature (°C)	0.04	0.07	0.20
1	10	0.17 ± 0.00^{Ac*}	0.18 ± 0.01^{Ab*}	0.18 ± 0.01^{Ab*}
2		0.17 ± 0.06^{Ac*}	0.18 ± 0.06^{Ab*}	0.18 ± 0.00^{Ab*}
3		0.18 ± 0.06^{Aabc*}	0.18 ± 0.06^{Ab*}	0.18 ± 0.00^{Ab*}
4		0.18 ± 0.01^{Abc*}	0.18 ± 0.00^{Ab*}	0.18 ± 0.01^{Ab*}
5		0.18 ± 0.01^{Abc*}	0.18 ± 0.01^{Ab*}	0.18 ± 0.00^{Ab*}
1	30	0.19 ± 0.00^{Aabc*}	0.20 ± 0.00^{Aa*}	0.20 ± 0.01^{Aa*}
2		0.20 ± 0.01^{Aab*}	0.20 ± 0.06^{Aa*}	0.20 ± 0.00^{Aa*}
3		0.20 ± 0.06^{Aab*}	0.20 ± 0.06^{Aa*}	0.20 ± 0.00^{Aa*}
4		0.20 ± 0.01^{Aab*}	0.20 ± 0.01^{Aa*}	0.20 ± 0.00^{Aa*}
5		0.20 ± 0.00^{Aab*}	0.20 ± 0.00^{Aa*}	0.21 ± 0.01^{Aa*}

Different capital letters in the same line indicate significant difference ($p < 0.05$) as a function of enzyme concentration. Different lowercase letters in the same column indicate significant difference ($p < 0.05$) as a function of temperature for each time. Different asterisks in the same column indicate significant difference ($p < 0.05$) as a function of time for each temperature.

Table 2. pH values of pasteurized goat milk incubated at 10 °C and 30 °C with different lactase concentrations (0.04%, 0.07% and 0.20% v/v) during 5 h.

Time (h)	Lactase %			
	Temperature (°C)	0.04	0.07	0.20
1	10	6.56 ± 0.06^{ABab*}	6.53 ± 0.06^{ABab*}	6.70 ± 0.10^{Aa*}
2		6.56 ± 0.06^{ABab*}	6.56 ± 0.06^{ABa*}	6.60 ± 0.00^{ABab**}
3		6.56 ± 0.06^{ABab*}	6.46 ± 0.06^{Ababc*}	6.53 ± 0.06^{ABabc**}
4		6.63 ± 0.06^{ABa*}	6.53 ± 0.06^{ABab*}	6.46 ± 0.06^{ABbcd**}
5		6.50 ± 0.00^{ABab*}	6.50 ± 0.10^{ABabc*}	6.40 ± 0.20^{Bbcd***}
1	30	6.40 ± 0.10^{Aabc*}	6.30 ± 0.10^{ABcd*}	6.30 ± 0.00^{ABde*}
2		6.27 ± 0.06^{ABcd*}	6.33 ± 0.06^{ABbcd*}	6.33 ± 0.06^{ABcde*}
3		6.27 ± 0.15^{ABcd*}	6.20 ± 0.00^{ABd*}	6.33 ± 0.06^{ABcde*}
4		6.33 ± 0.06^{ABbc*}	6.23 ± 0.06^{ABd*}	6.20 ± 0.00^{ABef**}
5		6.20 ± 0.20^{ABd*}	6.20 ± 0.10^{ABd*}	6.10 ± 0.00^{Bf***}

Different capital letters in the same line indicate significant difference ($p < 0.05$) as a function of enzyme concentration. Different lowercase letters in the same column indicate significant difference ($p < 0.05$) as a function of temperature for each time. Different asterisks in the same column indicate significant difference ($p < 0.05$) as a function of time for each temperature.

Table 3. Results of the physicochemical analyzes of lactose whole lactose (control) pasteurized goat milk incubated at 10 °C and 30 °C with different lactase concentrations (0.04%, 0.07% and 0.20% v/v) after 5 h.

%	Lactase %				Control
	Temperature	0.04	0.07	0.20	
Fat	10 °C	4.0 ± 0.25^{Aa}	4.1 ± 0.10^{Aa}	4.0 ± 0.20^{Aa}	3.8 ± 0.20^{Aa}
	30 °C	4.1 ± 0.20^{Aa}	4.0 ± 0.00^{Aa}	4.1 ± 0.30^{Aa}	
Proteins	10 °C	5.5 ± 0.21^{Aa}	5.5 ± 0.21^{Aa}	5.6 ± 0.40^{Aa}	3.9 ± 0.40^{Bb}
	30 °C	5.6 ± 0.10^{Aa}	6.0 ± 0.11^{Aa}	6.1 ± 0.20^{Aa}	
Casein	10 °C	89.9 ± 0.10^{Bb}	90.4 ± 0.20^{Bb}	91.2 ± 0.30^{Ab}	85.3 ± 0.30^{Cc}
	30 °C	90.4 ± 0.10^{Ba}	91.7 ± 0.15^{Aa}	91.9 ± 0.10^{Aa}	
Density	10 °C	1.043 ± 0.25^{Aa}	1.043 ± 0.10^{Aa}	1.044 ± 0.25^{Aa}	1.034 ± 0.25^{Bb}
	30 °C	1.043 ± 0.05^{Aa}	1.045 ± 0.00^{Aa}	1.046 ± 0.10^{Aa}	
[1]TDE	10 °C	15.7 ± 0.10^{Aa}	15.7 ± 0.00^{Ab}	15.8 ± 0.00^{Ab}	14.1 ± 0.00^{Bc}
	30 °C	16.0 ± 0.00^{Aa}	16.4 ± 0.00^{Aa}	16.4 ± 0.00^{Aa}	
[2]DDE	10 °C	11.7 ± 0.10 ^{Aa}	11.7 ± 0.10^{Ab}	11.8 ± 0.10^{Ab}	10.2 ± 0.10^{Cc}
	30 °C	11.9 ± 0.10 ^{Ba}	12.3 ± 0.10^{Aa}	12.3 ± 0.10^{Aa}	
Lactose	10 °C	0.40 ± 0.00^{Bb}	0.07 ± 0.00^{Db}	0.10 ± 0.00^{Cb}	4.3 ± 0.01^{Aa}
	30 °C	0.50 ± 0.01^{Bb}	0.08 ± 0.00^{Db}	0.10 ± 0.00^{Cb}	

[1]TDE: Total dry extract; [2]DDE: Degreased dry extract. Different capital letters in the same line indicate significant difference ($p < 0.05$) as a function of enzyme concentration. Different lowercase letters in the same column indicate significant difference ($p < 0.05$) as a function of temperature.

Samples added with 0.04; 0.07 and 0.20% lactase did not differ ($p > 0.05$) as a function of incubation temperature. This indicates that the refrigeration temperature (10 °C) can also be used at these concentrations of *Kluyveromyces lactis* yeast-β-galactosidase in pasteurized goat milk.

The lactose levels after treatment with 0.07% lactase and incubation at 10 °C and 30 °C were higher than those from samples with the addition of 0.20% lactase, therefore, demonstrating significant differences depending on the enzyme concentration ($p < 0.05$). Thus, it may be suggested that in such conditions enzymatic hydrolysis was lower with increasing lactase concentration from 0.07% to 0.20%. According to Sousa (2012), this is due to the high initial concentration of the enzyme that makes the substrate rapidly converted to galactose, a competitive lactase inhibitor, reducing its activity. In addition to inhibition by galactose, β-galactosidases from *Kluyveromyces lactis* are also inhibited by glucose according to the hydrolysis reaction product (Mateo et al., 2004).

The results obtained in this study using only the enzymatic hydrolysis process during the 5h period were satisfactory when compared to the findings of Antunes et al. (2014), which obtained 0.2 g/100 mL lactose in skimmed bovine milk subjected to enzymatic hydrolysis using 0.4 mL/L lactase from *Kluyveromyces lactis* for 21 h at 10 ± 1 °C, followed by the microfiltration process. Thus, enzymatic hydrolysis was enough to reduce lactose present in milk, eliminating other types of higher cost processes.

According to Brazilian legislation, foods considered lactose free must contain a quantity equal to or less than 0.1 g/100 mL lactose in the ready-to-eat product, while foods considered low in lactose must contain more than 0.1 g/100mL and less than or equal to 1g/100 mL (Brasil, 2017). The residual lactose results found in this study (Table 3) allow to include processed milk with 0.07% and 0.20% lactase in the lactose-free food category, except for milk incubated at 10 and 30 °C with the addition of 0.04% lactase, which can be classified as low lactose food.

Future studies will be carried out with the purpose of verifying possible changes resulting from enzymatic hydrolysis in the sensory attributes of goat milk pasteurized with hydrolyzed lactose. Therefore, projective techniques such as the qualitative technique of Word Association (AP) (Gambaro, 2018) and the preferential attribute elicitation methodology (PAE) (Soares et al., 2019) may be employed in the assessment of the level of consumer perception.

4 Conclusion

The degree of hydrolysis and total acidity of pasteurized goat milk increased with temperature. Reduction in lactose levels using low lactase concentrations resulted in an increase in protein levels, total casein, density, total and defatted dry extract. Therefore, combination of low lactase levels and hydrolysis at 10 °C promoted positive changes in lactose-free pasteurized goat milk. This study was the first reporting the changes resulting from enzymatic hydrolysis in the composition of pasteurized goat milk.

References

Altinçekiç, S. O., & Koyuncu, M. (2017). Yetiştirici Şartlarında Saanen x Kıl Melezi Keçilerinde Bazı Meme Ölçüleri ve Toplam Sağılabilir Süt Miktarı Arasındaki İlişkiler Üzerine Bir Araştırma. *Journal of Nature and Science*, 20(3), 227-234. http://dx.doi.org/10.18016/ksudobil.289456.

American Public Health Association – APHA. (2001). *Compendium of methods for the microbiological examination of foods* (4th ed.). Washington: APHA.

Antunes, A. E. C., Alves, A. T. S., Gallina, D. A., Trento, F. K. H. S., Zacarchenco, P. B., Van Dender, A. G. F., Moreno, I., Ormenese, R. C. S. C., & Spadoti, L. M. (2014). Development and shelf-life determination of pasteurized, microfiltered, lactose hydrolyzed skim milk. *Journal of Dairy Science*, 97(9), 5337-5344. http://dx.doi.org/10.3168/jds.2014-8020. PMid:25022681.

Association Official Analytical Chemists – AOAC. (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (12th ed.). Washington: AOAC.

Barlowska, J., Pastuszka, R., Rysiak, A., Król, J., Brodziak, A., Kędzierska-Matysek, M., Wolanciuk, A., & Litwińczuk, Z. (2018). Physicochemical and sensory properties of goat cheeses and their fatty acid profile in relation to the geographic region of production. *International Journal of Dairy Technology*, 71(3), 699-708. http://dx.doi.org/10.1111/1471-0307.12506.

Beltrán, M. C., Morari-Pirlog, A., Quintanilla, P., Escriche, I., & Molina, M. P. (2017). Influence of enrofloxacin on the coagulation time and the quality parameters of goat's milk yoghurt. *International Journal of Dairy Technology*, 71(1), 105-111. http://dx.doi.org/10.1111/1471-0307.12388.

Bosso, A., Morioka, L. R. I., Santos, L. F., & Suguimoto, H. H. (2016). Lactose hydrolysis potential and thermal stability of commercial β-galactosidase in UHT and skimmed milk. *Food Science and Technology (Campinas)*, 36(1), 159-165. http://dx.doi.org/10.1590/1678-457X.0085.

Brasil. Ministério da Saúde da Agricultura, Pecuária e Abastecimento. (2000). Regulamento Técnico de Produção, Identidade e Qualidade do Leite de Cabra (Instrução Normativa n° 37, de 12 de setembro de 2000). *Diário Oficial [da] República Federativa do Brasil*.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. (2001). Regulamento Técnico sobre os Padrões Microbiológicos para Alimentos (Resolução-RDC nº 12, de 02 de janeiro de 2001). *Diário Oficial [da] República Federativa do Brasil*.

Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. (2017). Altera a Portaria SVS/MS nº 29, de 13 de janeiro de 1998, que aprova o regulamento técnico referente a alimentos para fins especiais, para dispor sobre os alimentos para dietas com restrição de lactose (Resolução - RDC nº 135 de 8 de fevereiro de 2017). *Diário Oficial [da] República Federativa do Brasil*.

Burgner, E., & Feinberg, M. (1992). Determination of mono- and disaccharides in foods by interlaboratoty study: quantification of bias components for liquid chromatography. *Journal of AOAC International*, 75(3), 443-464. http://dx.doi.org/10.1093/jaoac/75.3.443.

Chaves, M. A., Souza, A. H. P., Colla, E., Bittenccourt, P. R. S., & Matsushita, M. (2018). Influences of chia flour and the concentration of total solids on the characteristics of 'dulce de leche' from goat milk. *Food Science and Technology (Campinas)*, 38(Suppl. 1), 338-344. http://dx.doi.org/10.1590/1678-457x.22017.

El-Kader, A. S. S. A., El-Dosouky, A. M., Abouwarda, A., All, S. M. A., & Osman, M. I. (2012). Characterization of partially purified β-galactosidase from *Bacillus subtilis*. *Journal of Applied Sciences Research*, 8(4), 2379-2385.

Fangmeier, M., Kemerich, G. T., Machado, B. L., Maciel, M. J., & Souza, C. F. V. (2019). Effects of cow, goat, and buffalo milk on the characteristics of cream cheese with whey retention. *Food Science and Technology (Campinas)*, 39(Suppl. 1), 122-128. http://dx.doi.org/10.1590/fst.39317.

Gambaro, A. (2018). Projective techniques to study consumer perception of food. *Current Opinion in Food Science*, 21, 46-50. http://dx.doi.org/10.1016/j.cofs.2018.05.004.

Hadjimbei, E., Botsaris, G., Goulas, V., Alexandri, E., Gekas, V., & Gerothanassis, I. P. (2020). Functional stability of goats' milk yoghurt supplemented with Pistacia atlantica resin extracts and Saccharomyces boulardii. *International Journal of Dairy Technology*, 73(1), 134-143. http://dx.doi.org/10.1111/1471-0307.12629.

Hodgkinson, A. J., Wallace, O. A., Boggs, I., Broadhurst, M., & Prosser, C. G. (2018). Gastric digestion of cow and goat milk, Impact of infant and young child in vitro digestion conditions. *Food Chemistry*, 245, 275-281. http://dx.doi.org/10.1016/j.foodchem.2017.10.028. PMid:29287371.

Horner, T. W., Dunn, M. L., Eggett, D. L., & Ogden, L. V. (2011). β-Galactosidase activity of commercial lactase samples in raw and pasteurized milk at refrigerated temperatures. *Journal of Dairy Science*, 94(7), 3242-3249. http://dx.doi.org/10.3168/jds.2010-3742. PMid:21700008.

Hourigan, J. A. (1984). Nutritional implication of lactose. *Australian Journal of Dairy Technology*, 39, 114-120.

Lucatto, J. N., Silva-Buzanello, R. A., Mendonça, S. N. T. G., Lazarotto, T. C., Sanchez, J. L., Bona, E., & Drunkler, D. A. (2020). Performance of different microbial cultures in potentially probiotic and prebiotic yoghurts from cow and goat milks. *International Journal of Dairy Technology*, 70(1), 144-156. http://dx.doi.org/10.1111/1471-0307.12655.

Mal, G., Singh, B., G. Mane, B., Sharma, V., Sharma, R., Bhar, R., & Dhar, J. B. (2018). Milk composition, antioxidant activities and protein profile of *Gaddi* goat milk. *Journal of Food Biochemistry*, 42(6), 1-8. http://dx.doi.org/10.1111/jfbc.12660.

Mateo, C., Monti, R., Pessela, B. C. C., Fuentes, M., Torres, R., Guisan, J. M., & Fernandez-Lafuente, R. (2004). Immobilization of lactase from *Kluyveromyces lactis* greatly reduces the inhibition promoted by glucose. Full hydrolysis of lactose in milk. *Biotechnology Progress*, 20(4), 1259-1262. http://dx.doi.org/10.1021/bp049957m. PMid:15296458.

Medeiros, G. K. V. V., Queiroga, R. C. R. E., Costa, W. K. A., Gadelha, C. A. A., e Lacerda, R. R., Lacerda, J. T. J. G., Pinto, L. S., Braganhol, E., Teixeira, F. C., de S. Barbosa, P. P., Campos, M. I. F., Gonçalves, G. F., Pessôa, H. L. F., & Gadelha, T. S. (2018). Proteomic of goat milk whey and its bacteriostatic and antitumour potential. *International Journal of Biological Macromolecules*, 113(1), 116-123. http://dx.doi.org/10.1016/j.ijbiomac.2018.01.200. PMid:29471095.

Mituniewicz-Malek, A., Zielinska, D., & Ziarno, M. (2019). Probiotic monocultures in fermented goat milk beverages - sensory quality of final product. *International Journal of Dairy Technology*, 72(2), 240-247. http://dx.doi.org/10.1111/1471-0307.12576.

Moreira, K. M. M., Coelho, L. H., Perini, C. C., Rapacci, M., & Karam, L. B. (2009). Produção de doce de leite com teor reduzido de lactose por β-galactosidase. *Revista Acadêmica Ciências Agrárias e Ambientais*, 7(4), 375-382.

Nakagawa, T., Ikehata, R., Myoda, T., Miyaji, T., & Tomizuka, N. (2007). Overexpression and functional analysis of cold-active β-galactosidase from *Arthrobacter psychrolactophilus* strain F2. *Protein Expression and Purification*, 54(2), 295-299. http://dx.doi.org/10.1016/j.pep.2007.03.010. PMid:17459724.

Nardi, J., Moras, P. B., Koeppe, C., Dallegrave, E., Leal, M. B., & Rossato-Grando, L. G. (2017). Prepubertal subchronic exposure to soy milk and glyphosate leads to endocrine disruption. *Food and Chemical Toxicology*, 100, 247-252. http://dx.doi.org/10.1016/j.fct.2016.12.030. PMid:28017703.

Özturk, H. I., & Akin, N. (2018). Comparison of some functionalities of water soluble peptides derived from Turkish cow and goat milk Tulum cheeses during ripening. *Food Science and Technology (Campinas)*, 38(4), 674-682. http://dx.doi.org/10.1590/1678-457x.11917.

Pradeep Prasanna, P. H., & Charalampopoulos, D. (2019). Encapsulation in an alginate goats' milk inulin matrix improves survival of probiotic Bifidobacterium in simulated gastrointestinal conditions and goats' milk yoghurt. *International Journal of Dairy Technology*, 72(1), 132-141. http://dx.doi.org/10.1111/1471-0307.12568.

Ramón, A. N., De La Vega, S. M., Ferrer, E. C., Cravero Bruneri, A. P., Millán, M. P., Gonçalvez De Oliveira, E., Borelli, M. F., Villalva, F. J., & Paz, N. F. (2018). Training small producers in good manufacturing practices for the development of goat milk cheese. *Food Science and Technology (Campinas)*, 38(1), 134-141. http://dx.doi.org/10.1590/1678-457x.02017.

Ranadheera, C. S., Evans, C. A., Baines, S. K., Balthazar, C. F., Cruz, A. G., Esmerino, E. A., Freitas, M. Q., Pimentel, T. C., Wittwer, A. E., Naumovski, N., Graça, J. S., Sant'Ana, A. S., Ajlouni, S., & Vasiljevic, T. (2019). Probiotics in goat milk products: Delivery capacity and ability to improve sensory atributes. *Comprehensive Reviews in Food Science and Food Safety*, 18, 867-882.

Rosolen, M. D., Gennari, A., Volpato, G., & Volken de Souza, C. F. (2015). Lactose hydrolysis in milk and dairy whey using microbial β-galactosidases. *Enzyme Research*, 2015, 1-8. http://dx.doi.org/10.1155/2015/806240. PMid:26587283.

Sales, D. C., Rangel, A. H. N., Urbano, S. A., Tonhati, H., & Galvão, J. G. B. Jr. (2018). Buffalo milk composition, processing factors, whey constituents recovery and yield in manufacturing Mozzarella cheese. *Food Science and Technology (Campinas)*, 38(2), 328-334. http://dx.doi.org/10.1590/1678-457x.04317.

Santos, W. M., Nobre, M. S., Cavalcanti, M. T., dos Santos, K. M. O., Salles, H. O., & Alonso Buriti, F. C. (2019). Proteolysis of reconstituted goat whey fermented by *Streptococcus thermophilus* in co-culture with commercial probiotic *Lactobacillus strains*. *International Journal of Dairy Technology*, 72(4), 559-568. http://dx.doi.org/10.1111/1471-0307.12621.

Shabbir, U., Huma, N., & Javed, A. (2019). Compositional and textural properties of goat's milk cheese prepared using *dahi* (yogurt) as the starter culture. *Brazilian Journal of Food Technology*, 22, e2018289. http://dx.doi.org/10.1590/1981-6723.28918.

Soares, E. K. B., Silva, R., Silva, W. P., Kuriya, S.P., Maçaira, P. M., Oliveira, F. L. C., Silva, M. A. A. P., Pimentel, T. C., Freitas, M. Q., Cruz, A. G., & Esmerino, E. A. (2019). An intra-cultural investigation in Brazil using Coalho cheese and preferred attribute elicitation. *Journal of Sensory Studies*, 35(1), 1-10.

Sousa, B. F. M. (2012). β-galactosidases: *aplicação à produção de alimentos funcionais* (Dissertação de mestrado). Universidade de Lisboa, Lisboa.

Trevisan, A. P. (2008). *Influência de diferentes concentrações de enzimas lactase e temperaturas sobre a hidrólise da lactose em leite pasteurizado* (Dissertação de mestrado). Universidade Federal de Santa Maria, Santa Maria.

Zolnere, K., & Ciprovica, I. (2017). The comparison of commercially available β-galactosidases for dairy industry. *Research for Rural Development*, 1, 215-222. http://dx.doi.org/10.22616/rrd.23.2017.032.

Appendix A. Supplementary tables.

Table A1. Experimental design.

Time (h)	Temperature (°C)	Lactase %		
1	10	0.04	0.07	0.20
	30	0.04	0.07	0.20
2	10	0.04	0.07	0.20
	30	0.04	0.07	0.20
3	10	0.04	0.07	0.20
	30	0.04	0.07	0.20
4	10	0.04	0.07	0.20
	30	0.04	0.07	0.20
5	10	0.04	0.07	0.20
	30	0.04	0.07	0.20

Table A2. Hydrolysis degree (%) of pasteurized goat milk with different lactase concentrations (0.04%, 0.07% and 0.20% v/v) incubated at 10 °C and 30 °C during 5h.

Time (h)	Lactase %			
	Temperature (°C)	0.04	0.07	0.20
1	10	$72.63^{Ch****} \pm 0.25$	$84.60^{Bh*****} \pm 0.10$	$92.60^{Ae****} \pm 0.20$
2	$74.73^{Cg****} \pm 0.21$	$85.63^{Bg****} \pm 0.21$	$94.43^{Ad***} \pm 0.40$	
3	$77.20^{Cf***} \pm 0.10$	$91.26^{Bf***} \pm 0.20$	$98.60^{Ac**} \pm 0.30$	
4	$80.73^{Ce**} \pm 0.25$	$95.80^{Bd**} \pm 0.10$	$99.90^{Aab*} \pm 0.25$	
5	$89.10^{Bd*} \pm 0.10$	$100.00^{Aa*} \pm 0.00$	$100.00^{Aa*} \pm 0.00$	
1	30	$92.60^{Cc****} \pm 0.20$	$95.10^{Be****} \pm 0.00$	$98.20^{Ac*****} \pm 0.30$
2	$97.90^{Bb****} \pm 0.10$	$97.93^{Bc***} \pm 0.11$	$99.30^{Ab****} \pm 0.20$	
3	$99.30^{Aa***} \pm 0.10$	$99.65^{Ab**} \pm 0.15$	$99.60^{Aab***} \pm 0.10$	
4	$99.65^{Aa**} \pm 0.05$	$100.00^{Aa*} \pm 0.00$	$100.00^{Aa*} \pm 0.10$	
5	$99.81^{Aa*} \pm 0.00$	$100.00^{Aa*} \pm 0.00$	$100.00^{Aa*} \pm 0.00$	

Different capital letters in the same line indicate significant difference ($p < 0.05$) as a function of enzyme concentration. Different lower-case letters in the same column indicate significant difference ($p < 0.05$) as a function of temperature. Different asterisks in the same column indicate significant difference ($p < 0.05$) as a function of time for each temperature.