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ORIGINAL ARTICLE

Retention of vitamin A after goat milk processing into cheese: a nutritional strategy

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Abstract The deficiency in micronutrients is a public health problem, principally in lower-middle-income countries. Vitamin A (VA) is considered a micronutrient fundamental to the maintenance and development of different tissues in the organism. Therefore, it is an essential micronutrient in the human diet. In these terms, goat milk is the leading food consumed to provide nutritional support in innumerous lower-middle-income countries. Here our work aimed to produce goat cheese studying strategies to promote the retention of VA. Our experiment design also explores the use of the salting process to evaluate the levels of VA retention. The level of VA in goat cheese was determined using LC–MS/MS analysis. Additionally, the redox status of the goat cheese in terms of lipid peroxidation and protein oxidation was determined. The texture analysis was also evaluated to verify if the redox status and salting process influence the texture profile. The results showed that the salting process during goat cheese production improves the retention of VA in goat cheese. Moreover, the salting process also is related to alterations in the status redox of the goat cheese and texture parameters. Therefore, our results show that goat cheese production can be an alternative to produced dairy derivates with recognized concentrations of VA for human nutrition.

Keywords Goat cheese · Vitamin a · Salting process · Goat milk

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Introduction

Vitamin A (VA) is an essential nutrient which represents a group of fat-soluble molecules (de Miranda Ramos et al. 2016). These molecules participate in different cellular processes that play an essential role in homeostasis and in cellular metabolism (Bastos Maia et al. 2019). Retinol and retinoic acid are the main actives metabolites of VA that can be found in different foods of human diet (Tozer et al. 2019). Even though it is an essential nutrient for human metabolism, the VA is not synthesized in the human body (da Cunha et al. 2019). Therefore, consumption of nutritional components containing VA metabolites is fundamental for the cellular development and maintenance of the human organism.

Milk and milk derivates products provide a rich font of vitamins for human consumption and nutrition (Cabiddu et al. 2019). Thereby, it is essential to reinforce that milk is a food with high nutritional value and widely consumed in the world (Lonnerdal 2016). To breastfeeding infants, the milk is usually the unique source of nutrients, vitamins, and act to protect against infections during early infancy (Henrick et al. 2017). In adult life, usually, the milk derivates, also known as dairy products, present higher consumption by the global market(Nascimento et al. 2019). In

the milk industry, the bovine milk predominates, but recently the goat milk importance has grown due to a significant increase in consumption (Pulina et al. 2018). Additionally, the production of goat milk also has increased, principally in South America.

Notably, most of the goat milk is produced by small farms, mostly in lower-middle-income countries (Pulina et al. 2018). This production, in part, is to produce goat cheeses. In lower-middle-income regions such as South America, the production of goat milk and dairy derivates are associated with domesticated goat animals(Aragao et al. 2019). Usually, these breeding occurs in regions where the animal feed is restricted, and, therefore, goat production is an alternative for farmers for economic income and nutritional survival. Another critical factor is that goats present high tolerance to heat stress, which corroborates to the breeding in more arid regions.

In South America, the goat milk and dairy derivates are usually produced in regions that present low levels of social-economic aspects. A common trend observed in these locations is the protein malnutrition and vitamin deficiency, especially VA deficiency(Kane et al. 2015). In these terms, it is known that VA deficiency can be associated with different alterations in the development of the organism. Additionally, VA is a fundamental micronutrient to keep the cellular homeostasis. In infants and children, the deficiency of VA can cause visual impairment and contribute to increasing the risk of illness (Dantas et al. 2018; Stevens et al. 2015). The deficiency of VA also is related to alterations in the central nervous system (CNS) development, and as a consequence, can induce cognitive impairments (Stevens et al. 2015). Northeastern Brazil region is the leading dairy goat producer, and usually, the production is only for local consumption. Additionally, this region in Brazil presents an elevated level of malnutrition, mainly deficiency in micronutrients such as VA (Lima et al. 2019).

Therefore, the objective of the present work was to evaluate the level of VA in goat milk and then study strategies to maintain VA after goat milk processing into cheese, as a mechanism of preservation of this essential micronutrient on a region of recognized VA deficiency in the population.

Materials and methods

Physicochemical characterization

The samples of the goat milk and goat cheese were characterized as previously described (Ambrosoli et al. 1988). The lactose was quantified according to method 984.15 of the AOAC International. The total protein content was quantified according to method 981.10 of the AOAC International, and pH was quantified according to method 981.12 of the AOAC International. The lipid content was determined by extraction with a cold solvent mixture according to a published method (Bligh and Dyer 1959).

Individual cheesemaking procedure

The goat cheese was manufactured using heat-treated (70 °C, 30 min) goat milk. Briefly, lactic acid was added with the milk (20 L) at 37 °C followed by the addition of calcium chloride (0.5 mL/L) and commercial rennet (0.8 mL/L; Ha-La®, Christian Hansen) and heated at 42 °C for 40 min. Then, the formed soft curd was cut into 1–2 cm cubes using an AISI 304 cutter, slowly stirred with a sterile glass (5 min) and homogenized. Thus, the curd was maintained in perforated containers (10 cm diameter) for 24 h at 7 ± 0.5 °C for whey removal. Then, cheese curd blocks were divided into two groups, one group of cheeses were salted through the immersion into a brine solution containing 20 NaCl (w/w) and kept at 20 °C for 2 h. The control goat cheese was prepared without salting and stored at 4 °C. Three cheesemaking trials were conducted, and cheeses were vacuum packaged in sterile polyethylene bags and stored for analysis.

Sample extraction

Liquid–liquid extraction with low-temperature purification was carried out, adding four aliquots of 2.5 mL of acetonitrile to 5.0 mL of milk sample, with constant stirring. The mixture was shaken on a shaking table for 20 min at 180 rpm. To this mixture, 2 g of sodium chloride was added (to promote the salting-out effect), followed by shaking until the dissolution of the salt (approx. 10 min on a shaking table at 180 rpm) and centrifugation at 2200 × g. The top phase was transferred to a 15 mL polypropylene centrifuge tube and kept in the freezer for 12 h at − 20 °C. After this time, the remaining liquid phase was transferred to a new 50 mL centrifuge tube and evaporated in a water bath (50–55 °C) under a gentle nitrogen flow until complete dryness. Finally, the dry extract was reconstituted with 1 mL of acetonitrile for further LC–MS/MS (Rubensam et al. 2011).

Liquid chromatography coupled to mass spectrometry (LC–MS/MS)analysis

Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to a triple quadrupole mass spectrometer SCIEX 5500 QTRAP (Foster City, CA, USA) was used for analysis. The separation was performed using a Zorbax Eclipse XDB-C18 column (C18 3.5 µm,

100 mm × 2.1 mm) coupled to a C18 guard column (Phenomenex, CA, USA). Injection volume was 2 µL, and the mobile phase in the gradient elution consisted of ultrapure water with 2 mM ammonium formate (A) and 2 mM ammonium formate acetonitrile, both containing 0.1% formic acid (B). The flow rate was 500 µL min^{-1}, and a three-minute equilibrating time was applied. The gradient started with 95% of A buffer decreasing to 5% at 1 min, then kept for 8 min and returned to initial conditions at 9.5 min. A diverter valve was employed to reduce the entry of matrix components in the spectrometer. Mass spectrometer resolution in multiple reactions monitoring (MRM) was unitary, and the dwell time applied was 50 ms. Fragment ions in MRM mode of each precursor protonated molecular ions were produced by collision-activated dissociation (CAD) in a collision cell. Nitrogen was applied as a nebulizer gas, curtain gas, heater gas, and collision gas. Collision gas (CAD) was set at high. Nebulizer gas (GS1) was set at 30 psi. Heater gas pressure and temperature were set at 55 psi and 700 °C, respectively. Electrospray capillary voltage was set at 5.5 kV. The collision energies were set at maximum for each transition. Quantification was performed by relating the peak area of analytes obtained via determination versus peaks from retinyl palmitate and retinyl acetate standards and expressed as µg/g wet weight for liver samples and µg/mL for plasma samples. All extraction procedures, handling of homogenates, and quantifications were performed in light-controlled areas to avoid photodecomposition of analytes (Kunzler et al. 2019; Rubensam et al. 2011).

Thiobarbituric acid-reactive species (TBARS)

As an index of lipid peroxidation, it was measured the formation of TBARS during an acid-heating reaction, which is widely adopted for the measurement of lipid redox state, as previously describe (Draper and Hadley 1990). In brief, the samples (0.1 g) were mixed with 0.6 ml of 10% trichloroacetic acid and 0.5 ml of 0.67% thiobarbituric acid and then heated in a boiling water bath for 25 min. TBARS were determined by absorbance in a spectrophotometer at 532 nm. Results are expressed as nmol of TBARS/mg of protein (Lowry et al. 1951).

Measurement of protein carbonyls

The oxidative damage to proteins was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described (Levine et al. 1990). In brief, proteins were precipitated by the addition of 20% trichloroacetic acid and re-dissolved in dinitrophenylhydrazine, and the absorbance was read in a spectrophotometer at 370 nm. Results are expressed as nmol of carbonyl/mg of protein.

Measurement of protein thiol content

Protein thiol content in samples was analyzed to estimate oxidative alterations in proteins (Ellman 1959). In brief, an aliquot of the samples was homogenized and was diluted in 0.1% sodium dodecyl sulfate, 0.01 M 5,50-dithionitrobis (2-nitrobenzoic acid) in ethanol was added, and the intense yellow color was developed and read in a spectrophotometer at 412 nm after 20 min. Results are expressed as mmol of SH/mg of protein.

Texture analysis

The goat cheese texture profile was determined using TA.XT2i texture analyzer (Stable Micro Systems Ltd., Surrey, UK). Samples were compressed in a dual cycle using a 36 mm cylinder probe with a maximum compression of 40% at a crosshead speed of 1.7 mm s^{-1}. Goat cheese hardness, cohesiveness, springiness, and adhesiveness were calculated from a force x time graph using the Texture Expert for Windows version 1 software (Silva et al. 2019). The analysis was performed for at least six replicates for each sample, and the mean and standard deviation was calculated.

Statistical analysis

The experiments were set up in a completely randomized design with three replicates. The results were expressed as the mean and standard deviation. Statistical analysis was performed using the software Graphpad Prism 7 and test T.

Results

Firstly, goat milk was characterized, focusing on the chemical composition and the redox status of the composition. In terms of protein, whole goat milk presents 3.12% (Table 1). The fat content was 3.36%, and the lactose content was 4.63%. Whole goat milk presented a total solid of 8.47%, and 0.70% of NaCl (Table 1). Considering VA, it was not detected any content of retinoids metabolites in goat milk (Table 1).

Moreover, two types of goat cheeses were produced. On one type, no salt was added, while on the other type, the salting process was used. After the maturation period, physicochemical characterization was performed on both kinds of cheeses (Table1). In terms of physicochemical parameters, it was observed that goat cheese produced under the salting process presented a higher value of

Table 1 Chemical characterization of goat milk and goat cheese

	Goat milk	Cheese + salt	Cheese
Protein (%)	3.12 ± 0.05	29.00 ± 0.18*	27.9 ± 0.20
Fat (%)	3.36 ± 0.06	25.13 ± 0.47*	22.0 ± 0.82
Lactose (%)	4.63 ± 0.08	ND	ND
pH	6.17 ± 0.59	6.27 ± 0.05	6.48 ± 0.01
Total solids (%)	8.47 ± 0.14	58.0 ± 1.44	55.4 ± 1.80
NaCl (%)	0.70 ± 0.03	0.68 ± 0.01	0.42 ± 0.01*
Retinol (ng/mL)	ND	3.87 ± 0.48*	1.27 ± 0.33

ND not detectable

*Different of cheese salt x cheese, $p < 0.05$

protein retention when compared with goat cheese that was not submitted to the salting process. Similar resultsre observed for fat when comparing both goat cheeses (Table 1). These results suggest that the salting process improves the retention of nutritional components when goat cheeses are produced using the methodology previously described.

Additionally, it was also observed that the levels of VA metabolites were 3.87 ng/mL in the cheeses submitted to the salting process and 1.27 ng/mL in the cheeses that did not receive the salting process treatment. These results demonstrated that VA metabolites could be preserved during the production of goat cheese, mainly when the cheese is submitted to the salting process. Moreover, VA concentration in the goat cheeses also reveals that goat cheese can be a good source of VA (Table 1), principally in low-income regions where the incidence of VA deficiency is high.

To check the quality of goat milk and the produced goat cheeses, the redox parameters of the goat milk and goat cheeses were determined. First, it was decided to evaluate the levels of lipid peroxidation. Our results showed that goat cheese submitted to the salting process presented higher levels of lipid peroxidation when compared with goat cheese without the salting process (Table 2). Additionally, the levels of lipid peroxidation were higher after goat cheese production than in natural goat milk. Considering proteins, the levels of protein carbonylation were determined. Both kinds of goat cheese presented higher levels of protein carbonylation when compared with goat milk (Table 2). Besides, goat cheeses also presented a decrease in the level of protein thiol content (Table 2). If taken together, our results suggest that during goat cheese production, alterations in redox status of compounds such as lipid and protein occur. These alterations can impact the quality of the final product, since alterations in lipid oxidation can alter the sensorial and physical properties.

Therefore, considering the level of oxidation observed, it was also decided to evaluate the instrumental textural properties of the produced goat cheeses. The instrumental texture properties determined were hardness, adhesiveness, cohesiveness, and springiness. Our results showed that the goat cheese submitted to the salting process presented lower hardness when compared with the other kinds of goat cheeses that were not submitted to the salting process (Table 3). Moreover, goat cheese submitted to the salting process presented higher cohesiveness than the goat cheese that not receive the salting process (Table 3)—our data suggests that the salting process is related to both chemical and physical alterations in the goat cheese.

Discussion

The goat milk is an outstanding nutritional food that presents intrinsic characteristics that are explored principally due to the health benefits (Park et al. 2019). Different authors have reported that goat milk can be denominated as a functional food due to the positive effects observed in the immunological system, inflammatory process, and have beneficial properties that reduce the effects of chronic diseases (Nachshon et al. 2019; Vandenplas et al. 2019). Additionally, goat milk is rich in micronutrients, such as VA. In terms of VA, it is important to reinforce that globally, VA deficiency is a severe public health problem, principally in infants and young children. Moreover, in low-income countries, poor diet corroborates with the prevalence of VA deficiency (Wirth et al. 2017). Here, our study observes that goat milk, when processed into cheese, presents significant content of VA. This finding could have a high impact in Brazil and especially in the Northeastern region of Brazil, where a high level of malnutrition, mostly related to micronutrients, exists. Different public programs

Table 2 Redox characterization of goat milk and goat cheese

	Goat milk	Cheese + salt	Cheese
TBARS (nmol/mg prot)	1.67 ± 0.04	5.72 ± 0.08[a]	3.67 ± 0.05[ab]
Carbonyl (nmol/mg prot)	4.52 ± 0.45	9.26 ± 0.67[a]	9.17 ± 1.18[a]
Thiol Content (μmol/mg prot)	34.39 ± 0.78	25.97 ± 0.38[a]	28.51 ± 1.03[a]

[a]Different from goat milk $p < 0.05$

[b]Different from Cheese + salt $p < 0.05$

Table 3 Mechanical properties of the goat cheese

	Cheese + salt	Cheese
Hardeness (N)	3.90 ± 0.31*	4.64 ± 0.17
Adhesiveness (N s)	0.16 ± 0.09*	0.06 ± 0.01
Cohesiveness	0.17 ± 0.01*	0.15 ± 0.01
Springiness	1.00 ± 0.01	0.99 ± 0.01

*Different $p < 0.05$

are conducted to reduce this public health problem, principally following the scope of the World Health Organization (WHO). Thus, the consumption of foods rich in VA can be considered as a possible mechanism to decrease the index of VA deficiency.

Especially in Northeastern Brazil, public programs use goat milk as a nutritional source to reduce the level of malnutrition and vitamin deficiency (dos Santos Souza et al. 2019). Additionally, dairy derivates from goat milk, such as cheese, are locally produced and consumed as an attempt to improve the dietary nutrient intake, principally by children attending schools (Dougkas et al. 2019). Nutritionally, goat milk is considered more digestible than other kinds of milk. Moreover, they have a different composition, principally in terms of fat and protein. These properties are also explored in dairy products such as cheese. Thus, a challenge for the production systems is the maintenance of nutrients composition in the dairy derivates. Our results reveal that cheese production from goat milk exhibits the potential to retain significant amount of VA in these dairy products. These results make clear that goat cheese production represents an alternative in low-income country's regions to improve the storage time of goat milk through the production of dairy derivates. Moreover, the production of goat cheese can be used as a strategy for readdressing malnutrition in low-income countries. Interestingly, our results also reveal that the salting process corroborates with the retention of VA in the cheese. Thus, the salting process mediates the physicochemical conditions that are responsible for altering the macromolecular structures of both protein and lipid molecules (Bansal and Mishra 2020). These alterations are also related to the retention of compounds in the cheese, independent of the type of cheese, and the source of the milk. Here, it is suggested that the salting process was able to alter the interaction protein–protein, mediating modifications in the casein interactions, and micelles formation.

Traditionally the salting method is used in the production process of generic cheese, and the use of sodium chloride is widely applied (Kaminarides et al. 2019). The antimicrobial activity, the ability to reduce water activity, and alter osmotic pressure are effects associated with sodium chloride in dairy products such as cheese (Estrada et al. 2019). Our results reveal that the use of sodium chloride was also associated with an increased level of lipid peroxidation, carbonylation of protein, and a decrease in protein thiol content of the goat cheese. These alterations reveal that the use of sodium chloride impairs the nutrient quality in the cheese.

The lipid peroxidation is associated with alterations in palatability, texture, and sensorial properties of the food products. Additionally, there are known toxic effects that oxidized lipids can promote in the cellular and physiologic metabolism. Lipid peroxidation is also related to the formation of carbonyl groups, mainly through the reaction of intermediaries of lipid peroxidation (Sottero et al. 2019). Thus, the lipid peroxidation can be a possible effect mediating the increase of protein carbonylation in the goat cheese that receives the salting method. The carbonylation of proteins is also related to changes in palatability, texture, and sensorial properties, principally in cheeses. The protein is the primary nutritional component in cheese, and alterations in protein structure can modulate the retention of micronutrients such as vitamins. The protein modifications both in covalent and non-covalent interactions contribute to the cheese structure and are responsible for some functionalities attributed to milk and their derivates such as allergenic properties (Li et al. 2019). Therefore, the use of sodium chloride in the salting method could present effects that usually are not intended in cheese products, and that usually are associated directly with milk quality or milk properties. However, considering VA, our results suggest that the salting process promotes the retention of this micronutrient in the goat cheese. Therefore, it can be used as a strategy to improve the concentration of this nutrient in the goat cheeses.

Even though the surprising results of VA retention obtained with the salting process, sodium reduction is still a challenge for the food industry, principally due to the recommendations given by the World Health Organization (Busch et al. 2013). In dairy products, sodium chloride is implicated in modulating the chemical and physical properties (Costa et al. 2019). The modulation of enzymes and alteration in the aroma are effects associated with sodium chloride, principally in cheese processes (Costa et al. 2019). Recent studies have shown that acceptance by consumers of dairy products with reduced salt content is satisfactory (Moatsou et al. 2019). Thus, our results corroborate the positive effects of salt in the retention of VA in goat cheese production.

Concluding, the results presented here reveal that the use of goat milk for cheese production can be a strategy to increase retention and improve the concentration of VA, principally in dairy products produced by low-income-countries where vitamin deficiency and malnutrition

represent a public health problem. Our results also reveal that goat cheese can be used as a good source for VA nutrition, principally in infants and children nutrition. These data reinforce that our findings may also be useful for improving the public health action and protocols using goat cheese as a source for keeping VA content within the normal physiologic range and the importance of carefully observe the outcome of vitamin supplementations in epidemiologic and experimental studies.

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