



Physicochemical composition, yield and sensory acceptance of Coalho cheese obtained from Zebu's cow milk



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Abstract:

The aims were to evaluate the effect of breed on the genetic polymorphism kappa-casein, physicochemical composition of milk and Coalho cheese, and on cheese yield; and to evaluate the effect of different periods of storage on sensorial acceptance of the Coalho cheese obtained from milk of Guzerat, Gyr and Sindi cows. Twenty (20) cows of Zebu breeds were selected, from which it was obtained the frequency values of the genetic polymorphism kappa-casein. Milk were submitted to fat, protein, lactose, non-fat solids and total solids, electrical conductivity analysis and somatic cell count. Cheeses were submitted to fat, protein, total solids, pH, moisture and yield (g TS/L) analysis. Attributes appearance, aroma, texture and flavor were judged at the 1st, 25th and 46th day of storage. There was a total frequency of 0.70 for genotype AA, 0.30 for genotype AB. There was no significant difference in milk composition among the studied breeds. However, there were differences in the physicochemical composition (with the exception of the protein) and the yield of the cheeses, but all the breeds showed a similar real yield. It was found effect of the storage period on the cheeses sensory attributes in the different breeds, with the exception of the appearance. The milk of the Guzerat, Gyr and Sindi breeds constitute an excellent raw material for the production of curd cheese and ensures a satisfactory sensorial acceptance of the product at the 1st, 25th and 46th days of storage.

Key words: *Bos taurus indicus*, Breed, Consumer, Dairy product, Storage.

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Introduction

Zebu cattle (*Bos taurus indicus*) were imported from India to Brazil in the 19th century. It represents more than 80 % of the national herd⁽¹⁾ due to its adaptability and performance in tropical climate conditions and has important participation in the success of cattle ranching in the country.

Zebu cows make up the bulk of the Brazilian dairy herd, including their crosses with specialized breeds (*Bos taurus taurus*) for milk production, especially the Dutch breed⁽²⁾. Brazil is the largest investor in genetic improvement of zebu cattle in the world⁽³⁾, involving strategic projects for genetic improvement of zebu animals with milk aptitude, mainly Gyr

and Guzerat. Current stock data for actions such as these show that the production means at 305 d of the Gyr, Guzerat and Sindi breeds are 11.25 kg⁽⁴⁾, 7.46 kg⁽⁵⁾, and 5.59 kg⁽⁶⁾, respectively.

Milk production and quality characteristics are directly influenced by environmental factors, nutrition, genetics and by the animal's own physiology⁽⁷⁾. Breed is a genetic factor with a relevant effect on the productive performance of dairy animals. The physiology of a specialized cow for milk production enables it to produce in large volume, but with low solids concentration, unlike a pure zebu cow with milk aptitude. This occurs because the production level of the cow is negatively related to the fat, protein and total solids percentages of the milk^(8,9).

Milk proteins can be classified into caseins and whey proteins. Caseins make up approximately 80 % of the milk proteins and are subdivided into 4 fractions: α_1 , α_2 , β and K. In the bovine species, genetic markers are used for selecting animals by determining gene pairs (A and B), which are present in milk caseins, such as kappa-casein. In general terms, the A allele has a significant effect on milk production and B allele on protein and fat concentration, resulting in a better yield of dairy products⁽¹⁰⁾. Several polymorphisms have been found for this protein, which is responsible for stabilizing the milk against heat treatments and clot formation⁽¹¹⁾.

The possibility of using milk to obtain dairy products is an important opportunity to add value to raw milk, diversify the product portfolio, and boost the competitiveness and profitability of the sector. Coalho cheese is a traditional dairy derivative of the culture of the Northeast Region of Brazil. It is a cheese obtained by a fermentation and coagulation process of raw or pasteurized milk. Cured cheeses from zebu breeds can already be found in the market, which shows the dairy potential of these breeds for cheese production. However, there are few studies on cheese obtained from zebu milk. Sensory evaluation is the most common method to analyze food quality⁽¹²⁾. The sensorial attributes of products can be measured via specific tests, identifying the importance of each of them for their acceptance by consumers⁽¹³⁾.

Therefore, the aims were to evaluate the effect of breed on the genetic polymorphism kappa-casein, physico-chemical composition of milk and Coalho cheese, and on cheese yield; and to evaluate the effect of different periods of storage on sensorial acceptance of the Coalho cheese obtained from milk of Guzerat, Gyr and Sindi cows.

Material and methods

Determination of the genetic polymorphism of kappa-casein

The steps between the DNA extraction and capillary electrophoresis were developed at the Central Gene Genetics Laboratory of Animal Genotyping Ltda (Belo Horizonte, Minas Gerais, Brazil) using in-house developed protocols.

The genomic DNA was extracted from the capillary bulb of each animal, producing a total of 22 samples. Buffered solutions containing a detergent and the tris-hydroxymethyl aminomethane (Tris), sodium chloride (NaCl) and ethylene diamine tetra acetic acid (EDTA) reagents were used for cell lysis.

After the DNA extraction, the samples were submitted to the polymerase chain reaction (PCR) technique of the STR regions, using a Veriti™ thermal cycler (Applied Biosystems, Forster City, CA, USA). Microtubes containing the necessary reagents for the enzymatic reaction were placed in the thermocycler: DNA fragments extracted from the capillary bulb, DNA-free water, deoxyribonucleotide triphosphates (dNTPs), oligonucleotide primers, DNA polymerase enzyme, magnesium and buffer solution. The primers used in the reaction were made by Life Technologies.

The amplified DNA fragments were subjected to capillary electrophoresis in a laser-induced fluorescence automated system (ABI Sequencer 3500xL) to verify the quality and concentration of DNA in each sample. The band reading was performed using GeneMapper Software®.

The fragments were induced to migrate by capillary electrophoresis, and then aligned based on size and detected by a laser beam. In the same run molecular weight standards and AA, AB and BB known samples were applied.

Finally, genotypic and allelic frequencies were obtained for the three evaluated breeds after identifying the genetic polymorphisms of the kappa-casein gene by the PCR technique.

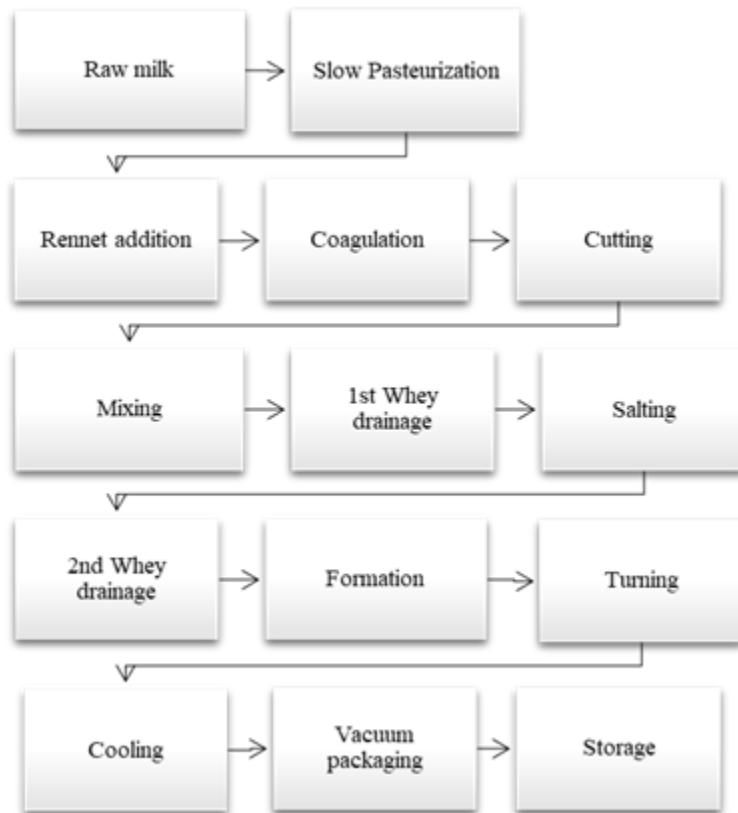
Raw material collection

Raw milk for producing Coalho cheese was obtained from Guzerat (n= 3), Gyr (n= 7) and Sindi (n= 10) females. The collection procedure for analyzing the physicochemical composition of the milk was performed manually using a properly sanitized stainless steel ladle after homogenization of the milk. The samples were conditioned in plastic bottles with a volume of 40 mL, identified individually, and kept in a thermal container with ice to preserve the temperature between 4 and 7 °C until the analysis procedure in the milk quality laboratory of the Federal University of Rio Grande do Norte. Fifteen liters of milk from each breed were also collected for producing Coalho cheeses, which were kept in isothermal containers and sent to the Dairy Processing Unit (DPU) of UFRN.

Coalho cheese production

Cheese production of the three breeds was carried out following the same technological manufacturing process, which was carried out at DPU of UFRN.

Milk samples from the three breeds for producing the cheeses were separately subjected to LTLT pasteurization (low temperature, long time 65°C/30 min). After thermal processing, they were cooled to 35 °C for rennet addition (Renin). After homogenization of the ingredients (milk and rennet), the mass was rested for 40 min until reaching the curd point, before cutting. The curd was subsequently heated under manual stirring to 45 °C. Then the whey was partially removed for salting the curd. The pre-pressing and forming procedures were carried out in the form itself for subsequent pressing and turning the curd. The cheese production process is shown in Figure 1. The process was finished with the vacuum cheese packaging and stored at 4 °C in a cooling chamber. The raw material, ingredients and packaging used for the cheese production were handled according to good dairy manufacturing practices.

Figure 1: Flow diagram for the production of Coalho cheese

Physicochemical analyzes of milk

The milk from the three breeds was analyzed for the fat, protein, lactose, solids-not-fat (SNF) and total solids (TS) percentages by the infrared absorption method in DairySpec FT[®] equipment (Bentley Instruments Inc., Chaska MN, USA). The electrical conductivity of the milk was measured using a Quimis[®] digital conductivity meter - ISO 9001 (SP, BR). The Somatic Cell Count (SCC) was estimated using the Somaticell[®] kit (Madasa, São Paulo, Brazil), following the manufacturer's recommendations. The SCC value varied from 69,000 cells/mL to 1'970,000 cells/mL.

Physicochemical analyzes of cheese

After producing the cheeses, 10 grams of each sample were removed and shredded in a Philipis Walita[®] blender (R12134) to reduce the particles, which were then submitted to physicochemical analysis of protein, fat, total solids, ash and pH. Protein percentage was determined based on the procedure of Cecchi⁽¹⁴⁾. The fat content was determined by extracting the petroleum ether solvent at 90 °C for 1 h using a Ankom[®] XT15 Extractor (NY, USA), following the equipment instructions. The percentage of total solids of the samples was established by the oven drying method at 105 °C for 6 h and the ashes determined by combustion of the organic matter in muffle furnace at 600 °C for 4 h⁽¹⁵⁾. The pH of the cheeses was determined using a previously calibrated Lucadema[®] 210 pH meter (SP, BR) with three readings per sample. All physicochemical analyzes of the cheeses were carried out at 46 d of maturation.

Calculation of Coalho cheese yield

The yield of cheeses was expressed in grams of total cheese solids per liter of milk (g TS/L) and calculated by the formula⁽¹⁶⁾:

$$Y \left(\frac{TS}{L} \right) = \frac{W \times ST \times 10}{V}$$

In which, Y= yield; W= kilos of cheeses obtained; TS= Total solids percentage of cheeses; V= milk volume used.

Sensory analysis

The sensorial acceptance test of the Coalho cheese samples was carried out at the Agricultural Sciences Unit - Jundiá Agricultural School (EAJ), Federal University of Rio Grande do Norte (UFRN) campus, conducted with 60 untrained female and male participants (18 to 60 yr old) who judged the attributes of appearance, aroma, texture and taste of the Coalho cheeses on the 1st, 25th and 46th days of shelf life. The evaluator selection was performed based on voluntary consent and the absence of allergic reactions to milk and dairy products. The sensory evaluation of Coalho cheese samples was carried out using a hedonic scale of 9 points, anchored at extremes 1 (I highly disliked it) and 9 (I liked it very much)⁽¹⁷⁾.

The tests were carried out by the participants individually in an environment with controlled humidity and temperature (air-conditioned room with air conditioning) in which white light was used, thus ensuring the ideal environmental conditions for conducting sensory analysis.

The participants were instructed on all the procedures for conducting the tests before beginning the evaluations. A small portion of a low-salt cracker and a portion of still water at room temperature was offered to be consumed between different samples to clean the palate and remove any residual taste. The Coalho cheese samples (25 g) intended for the tests were kept in ice isothermal boxes until they were served to the tasters in 50 ml white disposable plastic cups. Samples were coded with numbers composed of three random digits using a random number table.

Data analysis

Data analysis was performed using descriptive statistics by mean and standard deviation. The analysis of variance (ANOVA) of the data was performed to evaluate the effect of breed on the physical-chemical characteristics of the milk and cheeses, and on cheese yield. Each evaluator assigned their preference for the sensorial acceptance evaluations of cheeses by acceptability testing and the results were determined by means of the final average score of the scores presented by the judges to the different evaluated attributes in the sensorial analysis and submitted to analysis of variance (ANOVA). The Tukey test was used at 5% significance to compare the means of all analyzes using SAS software (version 9.0).

Results and discussion

Genetic polymorphism of kappa-casein

The frequency values of the kappa-casein genetic polymorphism in the Guzerat, Gyr and Sindi breeds are shown in Table 1. There was a total frequency of 0.70 (n= 14) for genotype AA, 0.30 (n= 6) for genotype AB, and 0 for genotype BB. No homozygous BB genotypes were found in this study. These results are in agreement with those reported in other studies, in which they showed a higher frequency of the AA and AB genotypes, and no observation of the BB homozygote in dairy breeds^(18,19).

Table 1: Distribution of the polymorphism frequency of the kappa-casein gene for the analyzed breeds

Breed	Kappa-casein polymorphism			Alleles	
	AA	AB	BB	A	B
Guzerat	0.66	0.33	-	0.83	0.17
Gyr	1	0	-	1	0
Sindi	0.50	0.50	-	0.75	0.25
Total	0.70	0.30	-	0.85	0.15

The highest frequency of the A allele in Brazilian zebu herds may be due to the origin of the animals and to selecting meat production at the beginning of their exploitation⁽²⁰⁾, since Indian zebu animals have a higher frequency of the B allele when compared to the Brazilian averages. Another factor is the number of animals at the effective herd level being selected. Homozygous animals are possibly being chosen for the A allele or using the heterozygotes in smaller proportions.

The polymorphism frequency of the kappa-casein gene for the B allele of the three breeds is close to that reported by others⁽²¹⁾. The authors analyzed the genetic polymorphism of kappa-casein in Brazilian zebu animals and found a frequency of 30 %, 1-10 % and 18 % of B allele in Sindi (n= 55), Gyr (n= 150) and Guzerat (n= 69), respectively. The selection of AB or BB animals in the kappa-casein genotype is important for dairy derivative production, since the B allele correlates with milk chemical composition parameters, mainly fat and protein, and promotes an increase in yield and cheese quality⁽¹⁰⁾.

The cheese yield of cows with genotype BB is higher in comparison to the milk from AA cows, and variant B is determinant in the efficiency process in milk coagulation time. The kappa-casein BB gene pair is correlated to higher processing characteristics, where cows with BB genotype for kappa-casein obtain shorter coagulation time for cheeses, higher density curd formation due to smaller micelle size, as well as higher cheese yield in relation to the milk from cows with AA genotype for kappa-casein^(22,23). Thus, this variant can be used as a selection criterion in breeding programs on farms with a cheese-based purpose. The B allele also has a positive influence on milk protein and fat content^(24,25); however, as in the present work, some reserchers^(26,27) found no effect on the protein percentage produced in animals of different genotypes.

Confirming the mentioned studies, the Sindi breed obtained the highest frequency of the B allele (25 %) when compared to the other breeds. This result may have implied the highest percentage of fat and total solids and yield in the cheese obtained from milk of Sindi breed.

Physicochemical evaluation of milk from zebu cows

The Table 2 shows the means and standard deviation for the physicochemical composition of the milk of the three breeds. There was no significant difference ($P>0.05$) for milk composition among the studied breeds. Similar results between the breeds can be attributed to the same management conditions employed and the similar genetic potential for milk composition.

Table 2: Physicochemical composition of the milk from the Guzerat, Gyr and Sindi zebu breeds

Item	Breed			P-value
	Guzerat	Gyr	Sindi	
Fat, %	5.14 ± 1.08	4.81 ± 0.67	5.35 ± 1.06	0.14
Protein, %	3.12 ± 0.48	3.13 ± 0.34	3.16 ± 0.37	0.24
Lactose, %	4.66 ± 0.71	4.68 ± 0.51	4.72 ± 0.56	0.21
SNF, %	8.51 ± 1.29	8.52 ± 0.92	8.60 ± 1.02	0.12
TS, %	14.16 ± 1.8	13.98 ± 1.41	14.65 ± 1.79	0.18
SCC, 10 ³ /mL	333.33 ± 348.53	243.10 ± 248.77	256.87 ± 444.65	0.62
ELC, mS/cm	3.94 ± 0.33	4.07 ± 0.35	3.81 ± 0.32	0.34

SNF= Solids-Not-Fat; TS= Total solids; SCC= Somatic cell count; ELC= Electrical conductivity.

Because there is no difference between the breeds, especially in fat and protein percentages, there is similar potential of the three breeds to produce these components. The total solids concentration in milk stands out as the main basis for paying for quality in most countries with a high development rate and in some places in Brazil.

In studying electrical conductivity (ELC) and somatic cell counts (SCC) of zebu cow milk, Moura *et al*⁽²⁸⁾ found higher values than those reported in the present study, which found 1'629,000 cells/mL for the Gyr breed and 1'356,000 cells/mL for the Guzerat breed, but the results found for ELC are close with results of 3.88 and 3.59 mS/cm for the Gyr and Guzerat breeds, respectively.

Physicochemical evaluation of dairy cheeses from zebu cows

Table 3 shows the mean values for physicochemical composition of the Coalho cheese from the Guzerat, Gyr and Sindi zebu breeds. The results demonstrate that the protein content was similar for the evaluated cheeses ($P>0.05$). The cheese from the Sindi breed

presented higher fat and total solids percentages, as well as a higher pH value when compared to those obtained from the milk of the other breeds. On the other hand, the Guzerat cheese obtained lower fat concentration and higher ash concentration, while the Gyr breed had a lower pH value. The fat percentage expressed in relation to the total solids avoids measurement errors in the yield occurring due to moisture loss. Described on a dry basis, the fat values of the cheeses respectively correspond to: 48.12 %, 53.79 % and 54.83 % for the Guzerat, Gyr and Sindi breeds. Thus, the results found are within those established by legislation for Coalho cheese⁽²⁹⁾, which defines between 35 to 60 % of fat in total solids as standard values. The regulation further states that Coalho cheese may be defined as semi-fat (25.0 to 44.9 %), fat (45.0 to 59.9 %) or extra fat (minimum of 60.0 %) in relation to fat content, and therefore the cheeses in this study are classified as fatty cheeses.

Table 3: Physicochemical composition and yield of Coalho cheese from the Guzerat, Gyr and Sindi zebu breeds (Mean + SD)

Item	Breed			P-value
	Guzerat	Gyr	Sindi	
Fat, %	24.26 ± 0.51 ^c	27.77 ± 0.73 ^b	32.23 ± 1.26 ^a	<0.05
Protein, %	18.77 ± 0.83	17.93 ± 1.39	17.91 ± 0.55	0.15
Total solids, %	50.41 ± 1.06 ^b	51.62 ± 0.07 ^b	58.78 ± 1.13 ^a	<0.05
Ashes, %	3.30 ± 0.01 ^a	2.58 ± 0.24 ^b	2.49 ± 0.30 ^b	<0.05
pH	6.92 ± 0.02 ^b	6.28 ± 0.05 ^c	7.16 ± 0.14 ^a	<0.05
Yield, g TS/L	82.25 ^c	83.33 ^b	93.68 ^a	<0.05

g TS/L: grams of total solids per liter.

^{abc} Means in the same line with different letters represent differences ($P < 0.05$).

The Sindi breed presented a higher percentage of total solids (TS) in the cheese, conferring greater potential for yield (g TS/L) in producing the derivative. There is still no regulation to standardize the physicochemical parameters of protein and ash, since the production process of most Coalho cheeses is still artisanal.

The pH values ranged from 6.28 to 7.16. These results were higher than those found by Araújo and Nassu⁽³⁰⁾ in evaluating the pH of industrialized and artisanal Coalho cheese, which varied from 5.10 to 5.80. The Sindi cheese had the highest pH (7.16).

The Sindi breed obtained higher ($P < 0.05$) performance in the Coalho cheese yield (g TS/L) due to the higher total solids concentration present in the milk. However, by analyzing the real yield (l/kg), all breeds obtained similar yield, using 6.13 (Guzerat), 6.05 (Gyr) and 6.27 (Sindi) liters of milk to produce 1kg of cheese, thereby confirming the potential of all breeds for cheese production.

Sensory evaluation of milk Coalho cheese from zebu cows

The results obtained from sensory analysis of the Coalho cheese of the three zebu breeds at different storage periods are presented in Table 4. The sensory scores varied from 6.32 (slightly liked) to 7.98 (moderately enjoyed). Coalho cheeses of different breeds presented similar appearance during the storage period ($P>0.05$).

Table 4: Sensory scores obtained on the acceptance test of Coalho cheese from zebu milk at different storage periods (Means \pm SD)

Breed	Day	Sensorial Parameters			
		Appearance	Aroma	Texture	Flavor
Guzerat	1	7.18 \pm 1.50	6.58 \pm 1.53 ^c	7.56 \pm 0.98 ^{abc}	7.70 \pm 1.27 ^a
	25	7.31 \pm 1.48	6.81 \pm 1.60 ^{bc}	7.55 \pm 1.18 ^{abc}	6.51 \pm 1.47 ^{bc}
	46	7.38 \pm 1.13	7.37 \pm 1.10 ^{abc}	7.71 \pm 0.99 ^{ab}	6.83 \pm 1.46 ^{bc}
Gyr	1	7.64 \pm 1.15	6.84 \pm 1.48 ^{bc}	7.85 \pm 1.02 ^a	7.98 \pm 1.03 ^a
	25	7.59 \pm 1.23	7.67 \pm 1.01 ^a	7.16 \pm 1.53 ^{abc}	6.44 \pm 1.49 ^c
	46	7.63 \pm 0.98	7.58 \pm 0.99 ^{ab}	7.05 \pm 1.32 ^{bc}	6.57 \pm 1.44 ^{bc}
Sindi	1	7.60 \pm 1.26	6.72 \pm 1.56 ^c	7.46 \pm 1.19 ^{abc}	7.28 \pm 1.47 ^{ab}
	25	7.36 \pm 1.28	6.91 \pm 1.51 ^{abc}	6.96 \pm 1.33 ^c	6.32 \pm 1.41 ^c
	46	7.52 \pm 1.15	7.19 \pm 1.47 ^{abc}	6.91 \pm 1.37 ^c	6.60 \pm 1.63 ^{bc}
p-value		0.12	<0.05	<0.05	<0.05

^{abc} Means in the same column with different letters are different ($P<0.05$).

On the first day of storage, it was observed that the cheeses obtained from the milk of the different studied breeds were similar in appearance, aroma and flavor ($P>0.05$), while only the texture of the Guzerat Coalho cheese (7.71) differed ($P<0.05$) from Sindi cheese (6.91) at 46 d of storage, reaching a higher score. The aroma of cheeses on the first day of storage had lower sensory scores (slightly appreciated), possibly due to the effect of coagulant proteolysis which may affect the availability of amino acids for enzymatic degradation⁽³¹⁾. Different aromatic compounds are generated throughout the storage period during cheese maturation due to several biochemical reactions^(32,33).

Cheeses made with cow milk from the Guzerat and Sindi breeds reached similar sensory acceptance ($P>0.05$) for the texture attribute during the whole evaluation period, while the cheese made with milk from Gyr cows showed the lowest acceptance (7.05) at 46 d of storage ($P<0.05$) than on the first day (7.85). According to Ordoñez⁽³⁴⁾, proteolysis causes changes in the texture and consistency of cheeses, which progressively loses its protein structure over the passage of time, thereby conferring greater softness. Another aspect to be

considered is that the Coalho cheese is characterized by the firm and “rubbery” consistency due to the aggregation of the fat molecules in the casein micelles, forming a kind of sponge, so that Coalho cheeses with a higher fat content, such as Sindi cheese, may be softer and less consistent, and thereby achieve lower sensory acceptance with these characteristics.

The cheeses received better ($P<0.05$) sensory scores (moderately liked) on the first day of shelf life for the flavor attribute. This is because the chemical composition of the cheese (fat, protein and lactose) influences the product’s taste, especially when there is maturation. This behavior occurs as a function of the lipases acting on the lipids, forming medium and short chain free fatty acids, esters, ketones and aldehydes, interfering in the sensorial characteristics of the cheese⁽³⁵⁾.

The consumer market is becoming more and more demanding with the aim to achieve more competitiveness and acceptance by consumers, and so the dairy sector has been seeking greater variety, improved quality and productivity. Products which reach long shelf life without affecting their sanitary, physicochemical and sensory properties are alternatives to boost wholesale and export trade.

Conclusions and implications

The milk from the Guzerat, Gyr and Sindi breeds presents favorable physicochemical characteristics for producing Coalho cheeses, obtaining yields higher than 40 %, therefore constituting excellent raw material for producing derivatives. In addition, the cheeses presented satisfactory sensorial acceptance during the studied storage periods.

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