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PARAMETERS OF PHYSICAL-CHEMICAL QUALITY IN MOZZARELLA FROM BOVINE AND BUFFALO MILK AS A FUNCTION OF SEASONALITY

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ABSTRACT

This present study aimed to determine the physical-chemical quality parameter of the cheese mozzarella produced from buffalo and bovine milk during the four seasons of the year. For that, the cheeses were obtained from two dairies located in the city of Itambé-Bahia and forwarded to the laboratory of Bromatology of the Faculty of Technology and Sciences - Campus Vitória da Conquista, for the analysis of moisture, ashes, proteins and lipids, according to methodology proposed by the Adolfo Lutz Institute (2008). The analysis were carried out in the period from 2015.1 to 2016.2, considering each season of the year. In the statistical analysis, the quantitative data were submitted to ANOVA. In order to compare the means between species, the K test was used and between seasons of the year the Duncan test (SAS, version 9.1). The differences were considered significant with $P < 0.05$. In general, the cheeses made from the bovine milk were more nutritious, when compared to the buffalo milk cheeses. In all evaluated parameters, the values differed among the cheeses ($P < 0.05$), except for the lipid content ($P > 0.05$).It was also observed the influence of the seasons on all the characteristics ($P < 0.05$). It is important to emphasize that the bromatological analysis is an important tool in guaranteeing a product of good quality made available for commercialization.

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INTRODUCTION

Milk and its derivatives associated with cheese consumption are products with properties that contribute to the consumer's health, since they present, in their composition, relevant contents of lipids, proteins, minerals and vitamins (Cao, Bradley and Ferrah, 2009; Teixeira; Bastianetto; Oliveira, 2005; Yuan; Yuan; Li, 2009a; 2009b). Cheeses are products derived from the coagulation of milk casein, with the retention of fat and salts in suspension, and the release of water, lactose and soluble salts as whey (Copatti and Pfuller, 2014). Among the most consumed dairy products, cheeses have stood out in Brazil and in the world, due to adaptations in their elaboration technique, and it made possible the rise of several cheese varieties (Magalhães, 2002). With more than 1,000 kinds of cheese produced in the world, the most found ones are: Danbo; Pategrás; Sandwich; Tybo; Roquefort; Gongorzola; Limburg; Fresh Ricotta; Minas; Prato; Batavo; Gouda; Edam; Emental; Fresh provolone; Silician; Fontina; Parmesan; Cheddar; Smoked cottage cheese; Grated; Cheese curds and Mozzarella (Melo, 2015). The growing consumption of the mozzarella cheese in Brazil and in the world, due to the fast food and pizzas eating habits, caused in 2004 an increase of 33% in its

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production in Brazil, which represented almost 145 thousand tons/Year (Santos, 2009b). The mozzarella cheese is smooth, non maturated, a stretched curd chesse, slightly salted, generally white or of a slightly yellowish color, with a shiny surface and found in different shapes and sizes (Cansian, 2005). Mozzarella cheese comes from the provinces of Salermo and Castile (Italy). The Brazilian legislation determines that the following denominations are valid: mozzarella, mozarela or muzzarella; The spelling "Mozarela" is the most employed one. Originally, the mozzarella cheese was made from buffalo milk. However, nowadays, the mozzarella is produced from several kinds of milk, with highlights to the bovine and buffalo ones. The mozzarella is probably the best known and most popular cheese on the European continent. It is also the most produced and consumed cheese in Brazil (Robert, 2007). According to the Food of Agriculture Organization (FAO), buffalo and bovine milk differ by its color, the former has a whitest visual appearance, however, butter and cheeses are equally white (FAO, 1991). Amaral, Carvalho, Silva and Brito (2005) emphasized the buffalo milk as presenting some peculiarities in comparison to the bovine milk, among them their sweet taste and opaque white color, which is also referred to by Macedo, Wechsler and Ramos (2001) and attributed the absence of β-carotene in buffalo milk. Milk and dairy products must have a quality standard for the population health, with their quality assessed through physico-chemical, microbiological and sensorial determinations. Minimum standards are required by the Ministry of Agriculture, Livestock and Food Supply (Venturoso, Almeida, Rodrigues, Damin& Oliveira, 2007).

In this sense, the present work aimed to determine the parameter of physical-chemical quality of the mozzarella cheese produced from the buffalo and bovine milk during the four seasons of the year and manufactured in the city of Itambé-Bahia.

MATERIALS AND METHODS

Sample collection: The samples of bovine and buffalo mozzarella cheese (the first is in a rounded shape and the second in a rectangular form) came fromtwo distinct dairy products stores located in the town of Itambé-BA. They were transported in their original containers, all kept refrigerated in a styrofoam box containing recyclable ice, following to norms recommended by the Adolfo Lutz Institute (2008), in order to maintain the characteristics of those cheeses. The cheeses were sent to the Bromatology Laboratory of the Faculty of Technology and Sciences - Campus in Vitória da Conquista-BA, for later analysis. The analysis were carried out during the period of 2015.1 and 2016.2, we considered each season of the year (Spring, Summer, Autumn and Winter) as relevant for this work.

Prepation of the sample: In each one, of the two dairy products store, we collected three different packages of cheeses (repetitions), and a triplicate of the samples was prepared to each one of the seasons corresponding to the period of accomplishment of this work. Initially, a homogenization of the samples was performed by using a homogenizer for further analysis.

Physicochemical analysis: The cheeses were analyzed for their moisture content, ashes, proteins and lipids according to the methodology proposed by the Adolfo Lutz Institute (2008).

Moisture analysis: The moisture content was determined by the direct method of gravimetry, through percentage (%) of greenhouse mass loss (Novaecnica @ NT 513) at 105ºC up to constant weight

Ash analysis: Ash content was determined by the incineration of the muffle furnace material (Neomed do BrasilLtda) at 550ºC or until the ashes got white.

Protein analysis: The quantification of the residual protein was determined by the macro-kjeldahl method. This method is based on three steps: Digestion, Distillation and Titration. In the digestion, the sample is heated with concentrated sulfuric acid until the carbon and hydrogen are oxidized. In order to increase the boiling temperature of the acid and increase the oxidation rate of the organic matter, a catalytic mixture is added to the reaction. During digestion, carbon is transformed into carbon dioxide (CO2) and hydrogen into water (H2O). The nitrogen of the protein is reduced and transformed into ammonium sulfate. In distillation, the aim of this step is to transform the nitrogen present in the solution in the form of ammonium sulfate (NH4 +) to gaseous NH3. By adding concentrated NaOH and heating the ammonia release occurs, and then it is separated from the mixture by distillation. The gas then reacts with a solution of boric acid, forming ammonium borate. Finally the titration. This step consists in the titration of ammonium borate with a standard sulfuric acid solution. The higher the volume of sulfuric acid spent in titration, the greater the amount of nitrogen present in the sample. The Nitrogen/Protein conversion factor was 6.38, this value was used for the conversion of milk and dairy products.

Lipid analysis: The total lipid content of the cheeses was determined by using the Soxlet method. This is based on the sample immersion in petroleum ether, which is coupled to lipid extractor (Marconi MA491).

Data analysis: The interpretation of the results was carried out based on the Normative Instruction from the Ministry of Agriculture and Supply and the Agrarian Reform, decree n° 146 (BRAZIL, 1996). In the statistical analysis, the quantitative data were submitted to ANOVA. For the comparison of means between species, we've used the K test and, between the seasons, the Duncan test (SAS, version 9.1). The differences were considered significant with $P < 0.05$.

RESULTS AND DISCUSSION

In general, the cheeses made from bovine milk were more nutritious, when compared to the ones made from buffalo milk. In all evaluated parameters, the values differed ($P < 0.05$), except for lipid content ($P > 0.05$). It was also observed the influence of the seasons of the year in all the characteristics ($P < 0.05$). For moisture content there was an amplitude of 12.90% for buffalo cheese and 7.50% for the bovine ones, which presents a general average of 44.74% and 46.45%, respectively (Table 1). According to decree No. 146 (Brazil, 1996), cheeses with moisture between 36.0% and 45.9% are considered of medium moisture, and cheeses with moisture between 46.0% and 54.9% are classified as of high moisture. In this sense, buffalo cheese is generally classified as of medium moisture and bovine cheese as of high moisture. When the individual samples were checked, we noticed that 33.33% of the buffalo cheese presented high moisture, whereas the bovine cheese presented a frequency of 55.55%. These

results evidenced the lack of standardization in cheese processing, which means that the criteria for its development is required. It is worth pointing out that all buffalo cheese samples with high moisture was found during Spring or Autumn. Similar situation was verified for bovine cheese, except for two high moisture samples found in other seasons, one in Summer and the other in Winter. Thus, the averages for moisture, in both cheeses, in Autumn and Spring were classified as of high moisture and in Winter and Summer as of medium moisture. Cheeses from both species presented similar moisture in Spring and Autumn (P> 0.05), however, the bovine cheese presented higher moisture in Summer and Winter (P <0.05). Cheeses made from buffalo milk presented higher moisture during the dry seasons. However, the typical buffalo mozzarella presented 57% for moisture, which makes possible a greater softness of the product (Castaldo, 1960). On the other hand, cheeses that mature at room temperature tend to dehydrate (Júnior *et al.,* 2014). However, chesses high moisture provides a reduction in the durability of the product, since there is a proliferation of pathogenic microorganisms at risk to human health (Pietrowski, Ranthum, Crozeta and Jonge 2008). According to Fachinetto and Souza (2010), this type of variation may result from the characterization failure at the moment of standardization of the milk being used. In a study conducted by Marino *et al.* (2010), he observed a moisture variation of 47.32% and 55.14% in eight brands of buffalo mozzarella cheese. Regarding Cecchi (2003), in a study carried out on theoretical and practical foundations in food analysis, the cheeses present moisture between 40 and 75%. In a study conducted by Rodrigues *et al.* (2011) around Goiânia-GO, they determined that the moisture content for the cheese was above 55%. Thus, the results found in this study with the variations according to the seasons are within the conformity of the literature and with the Ordinance No. 364/1997 (Brasil, 1997), where it is determined that the moisture content should be at most 60% for the mozzarella cheese in general. For ash levels we got 2.52% for buffalo cheese and 1.80% for bovine cheese, with a general average of 2.89% and 3.21% respectively, as shown in Table 2.

Marchiori (2006) analyzed the chemical composition for the mozzarella cheeses made from milk of the two species studied, then he determined the ash value for the buffalo cheese as 1.87% and 2.15% for the bovine cheese. Marino *et al.* (2010), in a study carried out with eight different brands of buffalo cheese, found a variation between 1.82% and 2.75%. Oliveira, Bravo and Tonial (2012), found a variation in the average ash content between 2.77% and 5.72% in the cheese samples. These results were similar to this present study. According to Cecchi (2003), we may have a variation of ash composition, since in cheeses and dairy products there are large amounts of calcium, phosphorus and chlorides due to the ingredients used in its production and the raw material used. The ash contents for dairy products varied between 0.7% and 6.0% (Cecchi, 2003). Cheeses from both species presented similar contents in Spring and Autumn, and superiority of bovine cheese in Summer and Winter. On the other hand, in each species, the bovine cheese had the highest ash content in Spring and Summer, while for buffalo cheese the content was significantly lower during the summer (P <0.05). The protein content presented a variation of 3.68% for buffalo cheese and 13.31% for bovine cheese. The values for the bovine cheese protein were higher than those found for buffalo cheese at all seasons (P <0.05). However, the seasons influenced the protein content only in buffalo cheese, with higher concentrations in Winter

Table 1. Moisture content of the cheeses according to the specie and the seasons of the year

	Buffalo	Bovine
Overall average	44.74b	46.45[a]
DP	2.83	1.80
Mínimum	41.7	43.1
Maximum	54.6	50.6
Autumn	46.90Aa	45.31aC
Winter	43.40Bb	48.54aA
Spring	46.15aA	44.59aC
Summer	42.49Bb	47.35aB

[a,b]Means followed by different lowercase letters in the row differ by the F-test at 5% significance.
[a,b]Means followed by different capital letters in the column differ by the Duncan test at 5% significance.

Table 2. Ash content for the mozzarella cheeses according to their kinds and seasons of the year

	Buffalo	Bovine
Overall average	2.89b	3.21[a]
DP	0.43	0.41
Minimum	2.04	2.02
Maximum	4.56	3.82
Autumn	3.11aA	3.01aB
Winter	2.78bAB	3.02aB
Spring	3.02aA	3.34aAB
Summer	2.63bB	3.49aA

[a,b]Means followed by different lowercase letters in the row differ by the F-test at 5% significance.
[a,b]Means followed by different capital letters in the column differ by the Duncan test at 5% significance

Table 3. Protein Theory of Sectional Cheeses and Sections of the Year

	Buffalo	Bovine
Overall average	14.67b	17.96[a]
DP	0.74	2.17
Minimum	12.64	7.27
Maximum	16.32	20.58
Autumn	14.88bAB	17.14aA
Winter	15.34bA	19.10aA
Spring	13.99bC	17.99aA
Summer	14.48bBC	17.63aA

[a,b]Means followed by different lowercase letters in line differ by the F test at 5% of significance
[a,b]Means followed by different capital letters in column differ by the Duncan test at 5% of significance

Table 4. Lipid content of the cheeses according to the species and seasons of the year

	Buffalo	Bovine
Overall average	11.94[a]	13.70[a]
DP	5.83	3.02
Minimum	3.19	5.87
Maximum	20.82	19.58
Autumn	5.93bC	16.35aA
Winter	15.66aB	14.73aA
Spring	18.50aA	11.61bB
Summer	7.66bC	12.11aB

[a,b]Means followed by different lowercase letters in line differ by the F test at 5% of significance
[a,b]Means followed by different capital letters in column differ by the Duncan test at 5% of significance.

and Autumn, followed by Summer and Spring. Table 3 shows the values found for protein determination. According to Andrade (2014), milk is altered by the diet given to the animal, as well as changes occur according to the climatic factors of the environment they live, which also influences the product standard. In a study conducted by Marino *et al.* (2010), the variation of protein content was 14.35% and 27.56% for the

mozzarella cheese made from buffalo milk. In an evaluation carried out by Marchiori (2006) on the chemical composition of cheeses made from milk of both species, the average protein was 24.70% for buffalo cheese and 21.74% for the bovine one. Vieira (2010), in a study about the mozzarella cheese made from milk with different somatic cells, found the average composition for bovine mozzarella cheese of 20.5%. The values found in this study are in accordance with the literature. Although Amaral *et al.* (2005) describe that the nutritional value and industrial yield of buffalo milk exceed cow's milk, little has been done to regulate standards of identity and quality standards for milk and dairy products of these species.

The fat content presented a variation of 17.63% for buffalo cheese and 13.71% for bovine cheese, those present a general average of 11.94% and 13.70%, respectively (Table 4). According to a decree No. 146 (Brasil, 1996), cheeses with fat contents between 10.0% and 24.9% are classified as lean. Although the cheese from both species were generally classified as lean, 47.22% and 5.55% of the buffalo and bovine cheese samples, respectively, had a fat content lower than 10.0%. All these samples were collected during Summer or Autumn for buffaloes and during Spring for bovines. The lipid contents were significantly higher in Winter and Autumn for bovine cheese and in the Spring for buffalo cheese ($P < 0.05$). During Autumn and Summer, the buffalo mozzarella cheese presented values of 5.93% and 7.66%, respectively. These levels were considered to be particular to the buffalo species, since fat presents a variable constituent that assumes values above 5.5% independently from the experimental conditions (Venturini, Sarcinelli& Silva, 2007; Rocha, 2008). During Spring, we got the value of 18.50%. According to INEMA (2016), Spring is a dry season and, according to Costa Filho *et al.* (2014), in dry seasons, the milk production gets lower, and it produces a greater concentration of fat in the mammary gland. The mean fat values found in the study conducted by Rodrigues *et al.* (2011), in the surroundings of Goiânia-GO, found the results of 20%. In a study done by Marino *et al.* (2010) found a great variation between the marks of 14.35% up to 27.56% .JalMarchiori (2006) found the values of 16.79% for bovine cheese and 18.68% for the buffalo cheese. Meanwhile, Castro (2012), in the evaluation of the physical-chemical and microbiological quality of mozzarella cheeses commercialized in the Ceasa[1] of Vitória da Conquista - BA, found values for total fat between 25.5% and 31.1%. This variation was also found in this present study, and Marino *et al.* (2010) explain that this type of variation occurs due to the type of milk used in the manufacture of the product.

Conclusion

The mozzarella cheese components varied according to the species, as well as the seasons of the year excepting the protein in the bovine cheese. The results found resemble, to a large extent, to those cited in the literature and in the relevant legislation. The buffalo milk cheese can be classified as lean and of medium moisture and the bovine milk cheese as lean and of high moisture. Therefore, the difference in the diet provided to the animal, during the different seasons of the year, significantly changes the milk, and it influences the product standard. The lack of cheese composition standardization characterizes a variability in its process, and it requires technology applications and/or specific standards aiming at obtaining the products that present the same quality during all periods of the year. It is important to emphasize the importance of initiatives and/or partnerships between the producer and the educational institutions that aim to promote greater knowledge to food producers, since the usage of bromatological analysis is an important tool in guaranteeing a product of greater quality made available for commercialization. Besides the bromatological analysis, we emphasize the importance of carrying out a production control through microbiological analysis, this guarantee the food safety, also assisting the monitoring process of cheese quality, and it adds value to the product. Therefore, complementary studies must be carried out in order to characterize the product as a whole, involving microbiological, texture and sensorial analysis of the commercialized chesses, and making them more suitable to the consumer's taste.

[1] CEASA is an open market where fruits and vegetables are sold.

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