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Research Article

Seasonal Variations Affect the Physicochemical Composition of Bufallo Milk and Artisanal Cheeses Produced in Marajó Island (Pa, Brazil)

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Abstract: This research was carried out to evaluate the influence of seasonal variation in physicochemical composition and microbiological profile of buffalo milk and of the artisanal cream and butter types cheeses produced on Marajó Island. Eighteen farms located of the island were involved in the study. Milk and cheese quality were evaluated in two typical seasons. The rainy, from January to June and dry, from July to December. Samples were submitted to a detailed set of laboratorial analyses. Physicochemical composition of buffalo milk was influenced by the seasons. Compared to rainy, the dry period showed an increase in fat (5.53±0.71 to 6.74±1.19) and lactose (4.77±0.20 to 5.20±0.20) concentrations and reduction in total solids (16.89±0.92 to 15.77±1.54), nonfat dry matter (9.94±0.36 to 8.99±1.23) and minerals (0.84±0.12 to 0.62±0.09) concentrations ($p<0.05$). The season influence was observed in physicochemical composition of both cheese-types. Compared to rainy season, in the cream cheese type, higher values ($p<0.05$) were found for titratable acidity (0.63±0.02 to 0.78±0.15), fat (32.38±3.22 to 36.89±4.59), fat in the dry matter (55.06±3.26 to 63.92±2.49) but lower values for water activity (0.99±0.02 to 0.49±0.03), minerals (2.37±0.55 to 1.40±0.02) and calcium (0.28±0.08 to 0.18±0.07). For the butter cheese type, in the dry season, higher values were found only for titratable acidity (0.49±0.02 to 0.38±0.0) and lower values for water activity (0.18±0.07 to 0.45±0.08). The study have shown that some components of the buffalo milk and artisanal cheeses produced on Marajó Island are influenced by the dry and rainy typical seasons and therefore, some sensory variations are expected around the year.

Keywords: Artisanal Marajó cheese, raw milk quality, seasonal variations

INTRODUCTION

The production of buffalo milk and dairy products have grown increasingly to appeal to consumers looking for differentiated nutritional and functional quality, besides special characteristics and taste (Araujo *et al*., 2012). This milk has elevated levels of fat, lactose, protein, Ca, Fe and P minerals, as well as A, C and B6 vitamins. In addition, it presents lower levels of vitamin E, riboflavin and cholesterol. Due to the absence of carotene and the presence of biliverdin (a green-blue pigment), as well as the presence of bioactive pentasaccharides and gangliosides, which are absent in cow milk, buffalo milk is the object of several revisions (Abd El-Salam and El-Shibiny, 2011; Araujo *et al*., 2012; Medhammar *et al*., 2012; Abd El-Salam and El-Shibiny, 2013).

Although the production of cow (83%), goat (2.2%) and sheep milk (1.3%) represent 87% of the world's production, buffalo milk is ranked second, with 13%, nearly 93 million tons/year (IDF, 2010), with an annual growth rate of 3.1%, over 1.8% the cow milk production. This estimate includes India and Pakistan with the largest variety of breeds and highest number of animals, with respectively 68 and 28% of the world production (IDF, 2010).

In Brazil, the buffalo population is 1.185 million, the largest herd of the species in the Americas, with the highest concentration (38.5%) in the state of Pará. Out of this amount, 263,088 animals are on the Marajó Island (IBGE, 2010), where, in several municipalities, the buffalo dairy activity is a relevant income factor for a large part of small and medium scale rural producers, contributing to their community in the region (Bernardes, 2007).

On the island, most of buffalo milk is used for artisanal cheese production, since the region is far from consumer centers and out of collection circuits. The most traditional cheeses are the cream cheese type and butter cheese type (Bernardes, 2007). The preparation process of these cheeses results from a centenary tradition passed down through generations, which contributes to maintaining the special characteristics of the product and is part of the culture of the population (Silva and Oliveira, 2003).

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According to technical regulation (Pará, 2013), Marajó Cheese is an artisanal product prepared in the geographical area of the archipelago of the same name, following historical and cultural tradition of the region. The cheeses are obtained by spontaneous coagulation, fusion and draining of curd of buffalo milk and/or buffalo added with bovine milk at a maximum proportion of 40%, which is washed with water and buffalo or bovine milk, then added with cream or butter. They have a soft, compact and closed texture, with small and few pores, pleasant aroma, greenish-yellow color (Simões *et al*., 2013); also, they are slightly acidic and salty and appropriate for immediate consumption (Pará, 2013).

At the high milk season, with nearly 12 tons/day volume, cheese production is estimated in 1.500 kg/day originating from nearly 60 dairies located mainly in the municipality of Cachoeira do Arari, Soure and Salvaterra, Arari region, Marajó Island (PA, Brazil) (SEBRAE, 2013).

Since the Marajó island is situated near the equator line, it presents a wet tropical climate with an annual mean temperature of 28°C, mean thermal amplitude of 4°C and relative moisture variations between 80 and 90% (Lima *et al*., 2005; Brazil, 2007). However, in this region, rainfall can be considered the main meteorological variable, with the seasonal rainfall regime, given that it has a rainy period, from January to June, in which the rain concentration is nearly 3000 mm, corresponding to 86% of the total annual rainfall; and a dry period, from July to December, with 487 mm, equivalent to 14% of this total (INMET, 2011). This climate variation causes buffalo milk production inconsistency throughout the year, following season and off-season periods, resulting from higher or lower availability of natural native pastures, the only food source for buffalos in Marajó.

The effects of seasonal variation in milk production and quality is important because it influences the quality of milk products, which depend on composition, which in turn varies according to location, lactation stage, breed and species, milking system, animal age and size, environment, climate, temperature and diet composition (Galvão *et al*., 2010). Hence, buffalo milk and dairy production and composition can be directly influenced by the season, as it affects food availability and quality for animals (Bastianetto *et al*., 2005). Several studies show the effects of seasonal variation on buffalo milk composition in different regions (Rangel *et al*., 2010, 2011; Araujo *et al*., 2011; Araujo *et al*., 2012).

Considering the socioeconomic and cultural importance of the production of artisanal Marajó cheeses and the factors influencing quality, this study aims to evaluate the effects of seasonal variation on physicochemical and microbiological composition of buffalo milk and artisanal cream cheese type and butter cheese type, produced on Marajó Island.

MATERIALS AND METHODS

Experimental design: The study was carried out in 18 dairies located in the municipalities of Soure, Salvaterra and Cachoeira do Arari, Arari region, in the Marajó Island, Northern extreme of Pará State (PA, Brazil), situated between parallels 0°38′ N and 1°55′ S and meridians 48°20′ W Gr and 51°57′. This region has great areas of natural pastures and flooding fields; therefore, it is adequate for milk and cheese production. In these three municipalities, the buffalos were maintained in natural pastures throughout the whole year, without food supplements in the dry season. The buffalo milk used in the preparation of the Marajo cheeses was obtained by manual milking.

Milk samples were collected in three municipalities. Samples of butter cheese type were collected in the municipality of Cachoeira do Arari and cream cheese type samples, in Soure and Salvaterra. The collections were carried out at two periods of time: in July of 2012, corresponding to the rainy season, harvest period and in December of 2012, corresponding to the dry season, off season.

Cheese manufacturing: Preparation of the artisanal cream cheese type and butter cheese type followed typical production procedures, traditionally used in the region:

- **Cream cheese type:** After morning milking, raw buffalo milk was partially skimmed in an electric centrifuge and submitted to spontaneous fermentation at a mean temperature of 28°C, for 24 h. Subsequently, the whey was manually removed and washed twice with water (2l water/1 kg curd mass) under heating (70°C, 15 min), for acidity reduction. A third wash with buffalo milk (2l milk/1 kg curd mass) was carried out, also under heating (70°C, 15 min); to fuse taste and components lixiviated by water, in other words, soluble solids. The paste was stretched on a table to reduce temperature to 40°C; then, it was pressed mechanically for removal of excess whey, cut into cubes and ground in a manual or an electric grinder, into small particles, assuming the appearance of meal. Salt (15 g/1 kg curd mass) and cream (1l cream/1 kg curd mass), obtained from the milk skimming, were added to the ground paste which was submitted to thermal treatment (80°C, 20 min) and continuous manual mixing. In this stage stretching occurred, the fused paste was distributed into Plastic Polypropylene (PP) packaging and cooled at room temperature (28°C).
- **Butter cheese type:** In the preparation of this cheese, raw milk was submitted to spontaneous fermentation at mean temperature of 28°C, for 24 h. The fermented cream, formed on the surface, was removed for butter production. The stages of

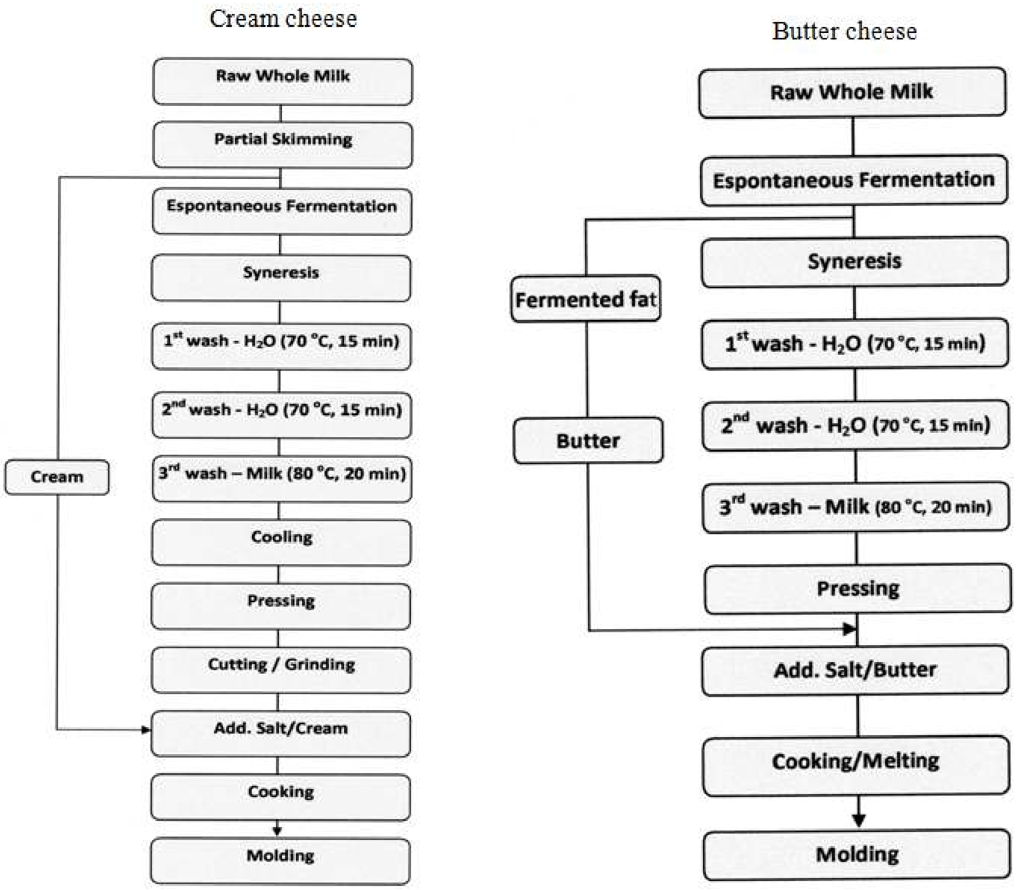

Fig. 1: Production flow chart of artisanal Marajo cream cheese type and butter cheese type

whey draining and washing with water and milk followed the same procedures as the cream cheese type. Subsequently, for removal of excess liquids, the paste was pressed manually. Salt (15 g/1 kg curd mass) and butter (100 g butter/1 kg curd mass), obtained from cooking the fermented fat were added and submitted to thermal treatment (80°C, 20 min) and continuous manual mixing. In this stage stretching occurred, the fused paste was then distributed into Plastic Polypropylene (PP) packaging and cooled at room temperature (28°C).

In each producing farm, three samples of each cheese type were selected and maintained under refrigeration (7-8°C). Figure 1 shows cheese production flow chart.

Buffalo milk and Marajó cheese samples: Samples of raw milk were collected at the end of milking, carried out in the morning, stored in sterile plastic bags and in standardized 40 mL plastic bottles containing Bronopol preservative (8 mg) for Somatic Cell Count (SCC). Cheeses of approximately 500 g, produced with this milk, were stored in plastic bags. Date, hour and place were marked on each sample at collection. All milk and cheese samples were transported to Belém (PA, Brazil) in isothermal boxes containing recyclable ice, at 4°C and submitted for laboratorial analyses. The physicochemical and microbiological analyses were carried out soon after the laboratories received the samples.

Physicochemical analyses: The variables analyzed in the buffalo milk were: pH, tritatable acidity, density, Cryoscopic Index (CI), protein, fat, lactose, Total Dry Extract (TDE), Nonfat Dry Extract (NDE), minerals and Somatic Cell Count (SCC). For cheeses, the following analyses were determined: pH, tritatable acidity, protein and fat, Fat Dry Extract (FDE), minerals, moisture, Water Activity (WA), chlorides, calcium and foreign material identification.

Cheese and milk fat was determined by the Mojonnier and Gerber methods, respectively (Brazil, 2006). Total protein content was estimated by the micro-Kjeldal method (Brazil, 2006). Moisture by drying was measured at 105°C to a constant weight (Brazil, 2006). Fixed mineral residue was measured by calcination in a muffle furnace (Brazil, 2006). Factor

6.38 was used for nitrogen conversion to protein. The pH values were measured by the potentiometric method (Metrohn Pensalab Analytical Instrumentation Ltda, Sao Paulo, SP, Brazil) and acidity was estimated by lactic acid percentage determination (Brazil, 2006). Total dry matter level was obtained by the sum of percentages of ashes, protein, lipids and carbohydrates (Kindstedt and Kosikowski, 1985). Determination of fat content in dry matter was carried out indirectly, by the ratio fat to total dry matter content of cheese (Pereira *et al*., 2001). Analysis of chlorides was carried out by the Mohr (1855) and Brazil (1981) and Water Activity (WA) by the instrumental method in digital meter (model AW43-Etec, Sao Paulo, SP, Brazil). Calcium concentration was determined by Atomic Absorption Spectrophotometry (Instituto Adolfo Lutz, 2008). Density (DE) 15°C (g/cm^3) and Cryoscopic Index (CI) were determined according to official methodology (Brazil, 2006). Analyses were carried out at the National Agricultural Laboratory, in duplicate. SCC was determined by the flow cytometry method, with automated equipment (Somacount, Bentley^R 300, Instruments Inc.), according to the International Dairy Federation (IDF, 2006), carried out in the Milk Quality Laboratory. Foreign Material identification (FMC) was performed by microscopy, by the acid hydrolysis technique, which was followed by the filtration described by the Association of Official Analytical Chemists, (AOAC, 2012), was carried out in the Public Health Central Laboratory of Pará State.

Microbiological analyses: Microbiological determinations for milk and cheese were carried out in the Public Health Central Laboratory of the State of Pará, following the valid legislation, as well as the standards of the Ministry of Agriculture, cattle-raising and Supply (MAPA). Standard Plate Count (SPC), Coliform count at 30°C, Coliform count at 45°C, *Staphylococcus aureus* count, *Samonella* sp., fungi and yeasts and *Listeria monoccytogene* were analyzed and determined according to techniques described by Instruction MAPA (Brazil, 2003).

Statistical analysis: To verify the effects of seasons on the physicochemical and microbiological composition in buffalo milk and Marajó cheeses, the averages of experimental data were submitted to two-tailed Student's t-test. The Coefficients of Variation (CV%) and Confidence Intervals (CI95%) were calculated so that variations of data in each analyzed season could be compared. In all analyses, 5% ($p \leq 0.05$) significance level was used. p-values were obtained by using BioEstat 5.0 software.

RESULTS AND DISCUSSION

Components of buffalo milk physicochemical composition, Somatic Cells Count (SCC) and Standard Plate Count (SPC), observed in the dry and rainy seasons is shown in Table 1.

Seasonality influenced buffalo milk composition (Table 1). In the dry period, an increase in fat and lactose concentration and a reduction in, Total Dry Extract (TDE), Nonfat Dry extract (NDM) and mineral levels ($p<0.05$) was noted. These differences can be sufficient to alter the sensory and technological properties of milk. The protein level, similar to physical variable results pH, acidity, DE and CI, did not occur significant alterations due to seasonal change ($p>0.05$) (Table 1). The physical and chemical results found in this study are in agreement with the Technical Regulations for Cooled Raw Milk Collection and Transport (Brazil, 2011).

Similar results were reported in Rio Grande do Norte (RN, Brazil), with fat content increase (5.27 to

Table 1: The effects of seasonality on physicochemical composition, somatic cell count and standard plate count of buffalo milk produced in Arari region, Marajó Island (PA, Brazil)

	Season of the year						
	Rainy (harvest season)			Dry (off-season)			
Variable	Mean±S.D.	CV%	CI95%	Mean±S.D.	CV%	CI95%	p-value
pH	6.870±0.170	2.47	6.790-6.940	6.860±0.120	1.82	6.810-6.900	0.8250
Acidity[a]	0.160±0.040	25.62	0.140-0.180	0.150±0.030	21.16	0.140-0.170	0.5780
DE	1.031±0.003	0.26	1.030-1.032	1.030±0.004	0.19	1.029-1.031	0.1670
CI	-0.565±0.051	9.11	-0.5405- -0.5887	-0.569±0.048	8.44	-0.5514- -0.5866	0.7550
Protein[b]	4.050±0.530	13.18	3.800-4.300	3.920±0.540	13.70	3.720-4.120	0.4110
Fat[b]	5.530±0.710	13.04	5.310-5.980	6.740±1.190	17.69	6.310-7.190	<0.0001
Lactose	4.770±0.200	4.23	4.690-4.840	5.200±0.200	3.93	5.100-5.290	<0.0001
TDE[b]	16.890±0.920	5.47	16.460-17.330	15.770±1.540	9.79	15.200-16.330	0.0021
NDE[b]	9.940±0.360	2.87	9.850-10.120	8.990±1.230	13.70	8.540-9.440	0.0001
Minerals[b]	0.840±0.120	13.07	0.800-0.900	0.620±0.090	13.89	0.590-0.660	<0.0001
SCC (x10^5)	2.900±2.200	73.81	1.900-4.100	2.400±2.400	100.18	1.100-3.700	0.4806
SPC[c] (x10^5)	2.320±1.500	64.53	1.570-3.060	1.400±1.370	97.60	0.720-2.080	0.0844

Statistical analysis: Student't test; Values represent mean±S.D. (n = 18); [a]: % lactic acid; [b]: g 100/g; [c]: UFC/mL; DE: Density (g/cm^3); CI: Cryoscopic index (°H); TDE: Total dry extract; NDE: Nonfat dry extract; SCC: Somatic cell count (cell/mL); SPC: Standard plates count; S.D.: Standard deviation; CV: Coefficient of variation; IC95%: 95% confidence interval

5.70%) in the dry season and similar concentrations of the other variables in both seasons (Araújo *et al*., 2011). However, the buffalos were fed in a pasture rotation system with sugarcane and urea-based supplementation, in the dry season. On the other hand, Araújo *et al*. (2012) and Andrade *et al*. (2011), in studies also carried out in Rio Grande do Norte (RN, Brazil) did not find significant alterations due to seasonal change on milk components, although fat concentrations in the dry and rainy periods (7.149-7.063%) observed by these authors were higher than those found in the present study.

In the Arari region, in dry periods animals grazed in natural pastures in arid areas, where forage is small and poor, with low nutritional value. Noticeably, the supply of complementary food was not usually used; consequently, milk production drastically decreased in this season (off-season); meanwhile a higher milk fat content was observed, but it did not reach expressive values (Table 1). The less milk volume in this period increased the solid/liquid volume ratio. Hence, the dilution effect can explain the fat concentration variation in the two seasons, which is also influenced by the lipid and fiber content of the ingested vegetation.

Fat value variations are expected, as it is the most sensitive milk component to a variety of factors, such as food management, genotype, nutrition, lactation, calving phase and order (Macedo *et al*., 2001). Fat, in both seasons, obtained the most elevated coefficient of variation, 13.04% in the dry and 17.69% in the rainy period, possibly indicating the use of pasture management and/or food supplementation in some farms. According to Fernandes *et al*. (2011), in studies carried out in Brazil, buffalo milk fat levels varied between 5.5 and 10.4%, with mean values nearly 6.0%. Thus, the milk fat concentration evaluated in this study is within the national average, though at the minimum established limit.

Protein concentrations, even relatively low, 3.72% (dry) and 4.05% (rain), range from 3.66 to 5.40% as reported by Amaral *et al*. (2005) and Teixeira *et al*. (2005), respectively. These values were lower than those reported by Bosquis *et al*. (2008) and Lopes (2009).

As fat is the major component in buffalo milk (Teixeira *et al*., 2005; Mattos, 2007), any modification in its concentration may influence total solid levels. However, in this study, in the dry period, the slight fat concentration increase was not able to neutralize the minimum lactose and mineral concentration values, causing a slight, but significant reduction in total solids concentration, with variation between 16.89 (rainy) and 15.20% (dry). In this study, TDE ranges from 15 to 17%, similarly to Amaral *et al*. (2005). However, these values were higher than the values obtained by Jorge *et al*. (2005), (13.88%), similar to those reported by Lamontagna and Franzolin (2009), (15.2%) and lower than those found by Oliveira *et al*. (2009), (18.3 and 20.12%), using supplementation with different lipid sources. Total solids are important parameters for industry, since their higher proportion in the milk indicates better yield in the manufacture of dairy. However, NDE, a less variable parameter, is used to standardize the milk solid level of herds in different handling conditions, as fat can vary up to 3% (Campanile *et al*., 2007).

Values of physical variables DE and CI were similar in both seasons and are in agreement with those reported by Teixeira *et al*. (2005), with DE between 1.025 and 1.047 g/mL and CI between -0.531 and -0.548°H, for the rainy and dry seasons, respectively. However, pH values found in the present study were higher than those reported by this author (6.41-6.47) but close to those observed by Finotelo (1981), who establishes pH values between 6.6 and 6.9 and between 7.00 and 7.06 by the end of lactation.

Lactose values were different from those reported by Araújo *et al*. (2011), 4.73% in the dry season and 4.82%, in the rainy season. However, these values are in agreement with those reported by Finotelo (1981), 3.30 and 5.90%, in rainy and dry seasons, respectively. In the present study, mineral concentration is within the interval of variation (0.75-0.85%) indicated by Finotelo.

The season change did not influence ($p>0.05$) Somatic Cell Count (SCC) (Table 1), for it was observed that during the rainy and dry seasons 66.6 and 70%, individual milk samples, respectively presented values below 2.5×10^5 cells/mL and only 38.8 and 30% showed count between 2.5×10^5 and 5.0×10^5 cells/mL. These values are in agreement with Normative Instruction number 62, which establishes, for the northern region, a maximum of 6.0×10^5 cells/mL (Brazil, 2011). The found SCC values are close to those reported by Andrade *et al*. (2011), with 2.5×10^5 and 1.9×10^5 cells/mL, in dry and rainy seasons, respectively.

A common parameter for evaluation of udder health, milk quality and monitoring of mastitis control programs is a SCC (Harmon, 1994), which is a direct indicator of the severity of mammary gland inflammation. Despite the myths about the buffalo species, these animals present similar sanitary problems as bovine, among them the occurrence of mastitis (Sollecito *et al*., 2011). Female buffaloes with elevated SCC present reduction of milk production (Cerón-Muñoz *et al*., 2002), alterations of component levels (Tripaldi *et al*., 2003) and coagulation time alterations during the cheese production process, compromising quality, processing and industrial yield (Singh and Singh, 1981). To avoid such alterations, it is important to maintain the threshold of somatic cells in up to 200.000 cells/mL (Tripaldi *et al*., 2003).

There is no evidence that SCC itself has any effect on human health. However, the presence of elevated counts presupposes the risk of raw milk being contaminated with pathogens and antibiotic residues,

Table 2: Effect of seasonality on physicochemical composition[a] of cream cheese type and butter cheese type, produced in Arari region, Marajó Island (PA, Brazil)

		Season of the year						
		Rainy (harvest season)			Dry (off-season)			
Cheese		Mean±S.D.	CV%	95% CI	Mean±S.D.	CV%	95% IC	p-value
Cream cheese type	pH	5.26±0.15	4.91	5.13-5.80	5.49±0.38	6.84	5.17-5.81	0.5138
	Acidity[b]	0.63±0.02	19.98	0.62-0.64	0.78±0.15	19.98	0.65-0.90	0.0002
	Moisture	41.26±3.48	8.43	38.35-44.17	40.29±1.98	4.91	38.63-41.94	0.1666
	WA	0.99±0.02	1.74	0.97-1.00	0.49±0.03	7.03	0.46-0.51	<0.0001
	Protein	24.99±1.63	6.53	23.63-26.35	23.08±2.16	5.49	21.27-24.89	0.0510
	Fat	32.38±3.22	9.96	29.69-35.07	36.89±4.59	12.43	33.05-40.73	0.0391
	FDM	55.06±3.26	5.93	40.73-57.79	63.92±2.49	3.90	61.84-66.00	<0.0001
	Mineral	2.37±0.55	23.08	1.91-2.83	1.40±0.02	1.40	1.38-1.42	0.0016
	Calcium	0.28±0.08	28.68	0.21-0.35	0.18±0.07	35.61	0.12-0.24	0.0387
	NaCl	1.18±0.11	9.61	1.08-1.27	1.21±0.20	17.11	1.04-1.38	0.7609
	(FMC)	39.00±28.14	71.34	15.47-62.53	10.98±4.50	41.06	7.22-14.74	0.0591
Butter cheese type	pH	5.18±0.06	1.19	5.11-5.24	5.39±0.41	6.44	5.04-5.39	0.0496
	Acidity[b]	0.38±0.02	5.40	0.36-0.40	0.49±0.02	16.57	0.47- 0.51	<0.0001
	Moisture	50.69±2.71	5.35	47.84-53.53	49.43±4.00	8.10	45.23-53.63	0.5355
	WA	0.99±0.02	1.62	0.97-1.01	0.59±0.08	13.13	0.51-0.67	<0.0001
	Protein	25.60±0.38	1.48	24.87-5.99	24.38±1.17	7.96	22.52-26.24	0.1920
	Fat	23.30±2.01	8.61	21.19-5.41	25.36±3.84	15.14	21.33-29.39	0.2710
	FDE	46.99±3.78	8.61	43.02-50.96	49.99±2.49	9.24	45.14-54.84	0.2460
	Mineral	2.68±0.03	1.15	2.65-2.71	1.40±0.02	0.74	1.41-1.43	0.0038
	Calcium	0.45±0.08	18.73	0.37-0.30	0.18±0.07	17.52	0.25-0.35	0.0255
	NaCl	1.51±0.16	10.65	1.34-1.68	1.21±0.20	16.00	1.12-1.58	0.1801
	(FMC)	73.02±23.97	32.64	52.98-93.06	60.00±8.46	14.14	52.93-67.07	0.2547

Statistical analysis: Student's t test; Values represent means±S.D., (n = 8); [a]: %, [b]: % lactic acid; WA: Water activity; FDE: Fat dry extract; FMC: Foreign material count; S.D.: Standard deviation; CV: Coefficient of variation (%); 95% CI 95%: Confidence interval

which may indirectly represent a risk to human health (Smith, 2002).

Standard Plate Count (SPC) results showed no significant statistical difference in the rainy and dry seasons (p>0.05). Besides, in both seasons, 100% of Buffalo milk samples were within the standards established by MAPA (Brazil, 2011). Although this Instruction is valid only for bovine milk, it was used in this study, given that in Brazil, there is no special legislation for buffalo milk. Normative Instruction N.62 only considers SCC and SPC as microbiological quality parameters for raw milk, not stipulating maximum allowed values for microorganisms considered in RDC51 (Brazil, 2002). If used RDC51, only 13.33% samples in the rainy season and 53.33% samples in the dry season would meet the microbiological standards established by legislation, as elevated counts of Coliforms were found at 30°C, as well as Coliforms at 45°C and *Staphylococcus aureus*. Contamination by *Salmonella* was not detected.

Table 2 shows mean physicochemical composition and foreign material count values found in cream cheese type and butter cheese type in rainy and dry seasons. Seasonal variation of buffalo milk composition influenced the final quality of Marajó cheeses. According to the results, six of the eleven physicochemical parameters of cheeses were influenced by the rainy and dry seasons.

In the dry season, cream cheese type and butter cheese type presented lower WA, mineral and calcium values (p<0.05), while acidity was higher. However, fat and FDE increased only in cream cheese type, in the same season. Conversely, there was no significant difference (p>0.05) for pH, moisture and protein and NaCl levels (Table 2). Hence, cream cheese type and butter cheese type produced in the dry season, which characterizes the off-season period, are safer and more acidic, however with less calcium, only the cream cheese type presented a more elevated level.

The results found in this study were not comparable to those obtained by Bittencourt (2011). This author evaluated seasonality only in Marajo cream cheese type, which did not alter its composition, except acidity, which significantly decreased in the dry season, in contrast to the present study. The values observed in that study, in the rainy and dry seasons, respectively, were lower for pH (5.14-5.15), acidity (0.68-0.57), fat (32.00-31.14%); FDE (56.48-55.84%) and protein (19.89-19.13%), but the values were higher for minerals (2.42-2.52%) and moisture (42.91-43.10%) (Bittencourt, 2011).

There is just one study with butter cheese type produced in the Marajó Island carried out by Finotelo (1981), but no study on the seasonal influence in this type of cheese. According to this author, medium composition typical of Marajó butter cheese type consists of the following: moisture, 37.38%; fat, 40.40%; FDE, 61.51%; protein, 22.7%; pH, 4.5; NaCl, 1.75%. It can be verified that values found here are higher for pH, moisture and protein and lower for fat, FDE and NaCl.

This current study shows that, besides being less acidic and salty, Marajó cheeses have similar protein concentrations but different fat concentrations in comparison to Finotelo's study. The butter cheese type is now produced with lower fat concentration (16.07% less) characterizing it as a healthier product. However, its quality may have been affected by this fat reduction, which is responsible for texture, paste delicacy and cheese taste. This is an important factor, which has a direct impact on the cheese quality appreciated by consumers. The cream cheese type, in contrast, has, today, nearly 12.64% more fat than when compared to the study of Finotelo (1981).

The increase in fat concentration found in the cream cheese type, in the dry period, may be related to the lack of standardization in the cream quantity added during the stretching of the curd mass and/or to the fat concentration increase in buffalo milk caused by milk volume reduction due to the reduction of pastures and quality. Conversely, for butter cheese type production, fat concentration increase of buffalo milk in the dry season did not influence fat increase; since during the stretching of this cheese, the reincorporation of great fat quantities in the form of butter is more difficult than cream reincorporation, in the cream cheese type. According to Toro and Sousa (2002), corroborated by reports of Nassu *et al*. (2001), fat concentration variations of milk used in the processing of cheeses affect curd formation and handling, as well as the ability to keep fat and moisture, influencing the composition of these dairy products.

Cream cheese type and butter cheese type were in accordance with the physicochemical standards established by the Technical Regulation for Marajó Cheese Production and by the Technical Regulation for Identity Determination and Quality of Cheese spread or *Requesón* (Pará, 2013). This guideline establishes that solid cheese spread must contain from 45 to 54.9% fat dry matter and maximum of 60% moisture, whereas for the butter cheese type spread, fat in the dry matter must be 25 to 59.9% minimum and maximum moisture of 58% (Brazil, 1997). Fat values classify cream cheese type and butter cheese type as semi-fat cheeses. Whereas moisture values classify the cream cheese type as middle moisture cheese while the butter cheese type is considered high moisture cheese (Brazil, 1997). Both have protein concentration in agreement with the established standards by the Ministry of Agriculture, Cattle-raising and Supply (Brazil, 1996), of 20 and 30%.

High Coefficient of Variation (CV%) values were obtained for acidity, minerals and calcium in the cream cheese type, in both seasons. CV% values for acidity show differences in the process used by producers, mainly concerning fermentation time, number of washings with water and milk and, possibly, the differences in the microbiotic compositions of raw buffalo milk, which depending on the lactic bacteria count and other present bacteria in the environment, can easily increase acidity (Queiroga *et al*., 2009).

In the rainy season, mean WA values were higher for both cheeses and are in agreement with Furtado, 1990, who establishes WA between 0.96 and 0.98 for cheeses with moisture between 40 and 49%, the range in which Marajó cheeses are. Thus, it can be stated that these cheeses are favorable medium for deteriorating and pathogenic microorganism growth, particularly *Staphylococcus aureus*, which may developed even at minimum WA of 0.86 (Jay, 2005). However, in the dry period moisture and salt concentration were not altered, but WA was reduced to half in both cheeses; this fact is possibly associated with an increase in the milk lactose concentration produced in this season, associated to not uniform manual pressing, which may have interfered in the quantity of lactose removed with the whey.

The high variation coefficients for minerals and calcium indicate differences in the animal diet and, possibly, supplementation carried out in some herds to supply the energetic and nutrient needs in the dry season. In addition, in the dry season less availability and quality of natural pastures, characterized by low mineral content, caused significant reduction ($p<0.05$) of mineral and calcium concentration in the buffalo milk and, consequently, in Marajó cheeses. Calcium levels in the cream cheese type (0.28-0.12%), in the rainy and dry period, respectively, were still lower than those found in butter cheese type (0.45-0.25%). Quantitatively, calcium represents the most important component in milk and its level can be influenced by the animal diet (Ferrara and Intrieri, 1974). Therefore, animal diet in the municipality of Cachoeira do Arari (PA, Brazil), a place where the butter cheese type is produced, could possibly justify this difference. The low calcium concentration in Marajó cheeses can also be a consequence of the production process, which involves acid fermentation, where high environment acidity increases mineral salt solubility, gradually changing to aqueous phase and calcium is among these minerals.

Mean microscopy results, in both cheeses, did not differ significantly in both seasons. However, it must be emphasized that high standard deviations and great Coefficients of Variation (CV%) found may have masked these results, mainly in the cream cheese type. The foreign material count average in the rainy season was 18% higher for the cream cheese type and 13% for the butter cheese type. When both cheeses are compared, in both seasons, it is noticed that the butter cheese type obtained higher physical contaminant rates, which indicate unsatisfactory hygienic habits during milking, milk filtration, processing and packing of cheeses and presence of animals in the adjacencies of the producing unit (Borsari, 2001). Inappropriate use of equipment and utensils in cheese processing increases the quantity of dark and brown spots, originating from earthen material, insect and leaf fragments suspended in the air and carried by the wind.

Table 3: Microbiological results of cream cheese type and butter cheese type produced in Arari region, Marajó Island (PA, Brazil), in both seasons

		Season of the year	
Cheese	Microbiological analysis	Rainy (harvest season)	Dry (off-season)
Cream cheese type	Coliform at 35°C[a]	<10	<10
	Coliform at 45°C[a]	<3	<3
	Samonella sp.[b]	Absence	Absence
	Coagulase-positive *Staphylococcus*[c]	<10	<10
	Listeria monoccytogenes[b]	Absence	Absence
	Fungi, yeasts[c]	120 a 130000	10 a 130000
Butter cheese type	Coliform at 35°C[a]	<10	<10
	Coliform at 45°C[a]	<3	<3
	Samonella sp.[b]	Absence-	Absence
	Coagulase-positive *Staphylococcus*[c]	<10	<10
	Listeria monoccytogenes[b]	Absence	Absence
	Fungi, yeasts[c]	1300 a 47000	800 a 45000

[a]: NMP/g; [b]: Absence in 25 g; [c]: UFC/g

During the processing of Marajó cheeses, bags made of cotton fabric, of easy fragmentation, are still used in several dairies for milk filtration and manual whey draining of the curd mass. Thus, presence of fabric fragments was found in 100% of the samples. Human and animal hair, the latter in higher quantity, as well as insect fragments were found in all analyzed samples, indicating nonuse of good manufacturing practices and physical precarity of producing units. These physical contaminants may carry pathogenic microorganisms associated with diseases such as salmonella, leptospirosis, black plague and typhoid, representing risk to the consumer (Fontes and Fontes, 2005). Similar results were found by Pimentel Filho *et al*. (2005) and Fernandes *et al*. (2011), in the microscopic analysis of fresh Minas cheese. The Table 3 shows microbiological evaluation results of Marajó cheeses, in both seasons.

Microbiological results of cream cheese type and butter cheese type were not affected by seasonality. Mean values for coliform count at 35°C, coliform count at 45°C, *Salmonella sp*., coagulase-positive *Staphylococcus*, *Listeria monoccytogenes* and fungi and yeasts (Table 3), were within the microbiological standards established by the Technical Regulation for Marajó Cheese Production (Pará, 2013), Technical Regulation of Identity and Quality of the Cheese spread (Brazil, 1997) and Technical Regulation on Microbiological Standards for Foods (Brazil, 2001).

These results indicated that thermal treatments applied during curd mass washings and stretching were able to reduce and almost remove the microbial load found in raw milk used in the production of these cheeses. However, in both seasons, elevated levels with great variations in fungi and yeasts count were detected in almost 90% of the butter cheese type samples and in nearly 40% of the cream cheese type samples. Similar results were reported by Sousa *et al*. (2002), Lourenço *et al*., 2002, who observed this contamination after the first manufacture day. Brazilian legislation does not stipulate fungi and yeasts limits for cheeses, but these microorganisms cause alterations in foods, since they produce enzymes that hydrolyze proteins, lipids and carbohydrates. This degradation creates compounds that promote modifications in color, appearance, loss of taste and toxic metabolites such as mycotoxins, which make the cheese unsuitable for consumption as well as reduce its shelf life (Franco and Landgraf, 1996).

Since these microorganisms can be widely distributed throughout the processing plant and/or via air circulation, overall hygiene of these variables is essential in order to prevent the recontamination of cheeses during the cooling and packaging process. This is especially true for the butter cheese type, which is generally wrapped in wax paper only. Thus, greater care and protection is recommended in these stages, as well as the use of vacuum packaging which creates an anaerobic environment averse to the growth of these microorganisms.

CONCLUSION

The results, besides confirming the importance of evaluation and routine quality monitoring of Marajó buffalo milk and cheeses, showed that dry and rainy typical seasons influenced the composition of buffalo milk and these differences were sufficient to alter the concentration of some constituents of artisanal cheeses produced in the region of Arari, Marajó Island, which could compromise consumer fidelity and consolidation of the producing region.

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