Journal of Bioanalysis & Biomedicine

Pignata et al., J Bioanal Biomed 2015, 7:6
DOI: 10.4172/1948-593X.1000143

Research Article | Open Access

Mechanical Parameters of the Mozzarella from Buffalo with Inclusion Levels of The Cow's Milk: Preliminary Study at the Lab Scale

Mirelle C Pignata, Sibelli Passine B Ferrão, Crisitane P Oliveira, Amanda S Faleiro, Renata C Bonomo, Willian S Silva, Luciano B Rodrigues and Sérgio Augusto de Albuquerque Fernandes*

Department of Rural and Animal Technology, State University of Southwest of Bahia, Brazil

Abstract

The aim of this study was to evaluate the inclusion of the cow milk into buffalo milk during cheese-making on their physical-chemical and mechanical properties. The cow milk was added (0%, 10%, 20%, 30%, 40% and 50%) into buffalo milk to produce the mozzarella. We evaluated the physical-chemical composition, textural profile analyzer and melt ability. The cow milk inclusion in the buffalo milk altered the L* value of the mozzarella, decreasing it, and the mozzarella chromaticity (a* and b*) linearly and positively. Those attributes (firmness, chewiness, elasticity and cohesivity) are affected with cow's milk inclusion, producing the lowest firmness and elasticity, characterized as less firm samples. The same behavior was observed for to melt ability, which decreased with cow milk inclusion. The measures of instrumental color, texture and the melt ability indicated that the cheese made with different levels of cow's milk presented distinct characteristics of the mozzarella from just buffalo milk. Instrumental tests as color, texture and melt ability can be used in the detection of the buffalo's mozzarella adulteration by cow's milk, showing a potential alternative for the quality control of the buffalo's mozzarella cheese.

Keywords: Texture profile analyses; Color; Melt ability

Introduction

The Mozzarella cheese is a pasta filata soft cheese originated in Italy, made traditionally with fresh buffalo milk and called "Mozzarella di Bufala Campana" with Protected Designation of Origin (PDO) [1-3]. Seeking to ensure the authenticity of buffalo mozzarella cheese in Brazil, the Brazilian Buffaloes Breeders Association (ABCB) launched in 2000 the purity seal for this cheese produced in this country [4]. For this label is printed on the packaging, the sample mozzarella should be submitted to the laboratory tests, such as electrophoresis, in order to identify the presence of bovine casein.

Factors such as: (i) a reduction in the production of buffalo milk in the summer, as a function of reproductive species seasonality [5]; mozzarella cheese scarcity in the market [6]; (iii) resulting in increase of the mozzarella price, has stimulated the frauds, with the mixture of cow's milk into buffalo's milk to make buffalo mozzarella. These versions are frauds that alter the mozzarella quality, breaking laws and consumers' rights [7]. Studies of texture reveal factors such as: functional, sensorial, structural, mechanical and superficial characteristics of the foods [8,9]. The texture is influenced by some factors such as: (I) proteolysis that break down the polypeptides casein chain; (ii) high fat content, which reduces cheese firmness; (iii) high moisture, which accelerates the protein hydrolysis; (iv) long time to stabilization and, (v) the use of the cultures with high proteolytic action.

The milk (heat treatment, composition), processing (final pH, acidification rate, among others), chemical composition of the cheese (pH, protein and fat content), mineral content (Ca and P total, total soluble Ca associated to casein), as well as the storage (time X temperature, residual plasmin, microbial activity, among others) affect the functional properties of cheese [10]. The sensorial analysis provides more accurate answers on the texture properties of foods [8]; however, the instrumental methods could be important alternatives to food texture evaluations, possibly serving as auxiliary tools for sensorial analysis [11].

Methods as immunological, electrophoretic and chromatographic have been proposed for the identification of buffalo milk adulteration [7,12,13]. However, the official control method, which is suggested by the European Union to detect bovine proteins in dairy products, relies on iso-electrofocusing (IEF) [14]. However, some researcher question results from this methodology [15] and recently Sakaridis, Ganopoulos, Argiriou and Tsaftaris (2013) suggested a new method based on DNA.

Among the various methods developed, as mentioned above, the analysis of the instrumental texture profile analysis (TPA) could be used as screening test. It consists on the use of successive deforming forces on the food test, simulating the compression of teeth during chewing [16] and provides information about the structure of the product. So, TPA could assist the industry in quality control of their products [17], because it's easy to use and has low cost. Even with the adoption of the seal of purity of the ABCB, used by a few industries, it is still possible to find on the market products derived from adulterated buffalo milk. We have many studies with buffalo's mozzarella (BM); however, neither was made about adulteration to the cow's milk. In this context, the aim of this study was to evaluate the inclusion of the cow's milk in the buffalo milk (mix) on the mozzarella by TPA.

Materials and Method

Samples

Fresh raw milk was obtained from the commercial herd (30 cross buffaloes Zaffarabadi X Murrah) and from the herd of the Universidade Estadual do Sudoeste da Bahia (30 cross cows Frisian x Zebu), mixed

***Corresponding author:** Sergio Augusto de Albuquerque Fernandes, Department of Rural and Animal technology, State University of Southwest of Bahia, Itapetinga-BA, Brazil, Tel: +773-261-8629; E-mail: sfernandes@uesb.edu.br

Received July 10, 2015; **Accepted** November 30, 2015; **Published** December 03, 2015

Citation: Pignata MC, Ferrão SPB, Oliveira CP, Faleiro AS, Bonomo RC, et al. (2015) Mechanical Parameters of the Mozzarella from Buffalo with Inclusion Levels of The Cow's Milk: Preliminary Study at the Lab Scale. J Bioanal Biomed 7: 191-196. doi:10.4172/1948-593X.1000143

respectively, at morning, from 2012 April-June. For each repetition 30 L of milk was used. The experimental design used was random block design, with six treatments (cow's milk levels inclusion) at 0, 10, 20, 30, 40 and 50% for manufacturing of the mozzarella, with three repetition and three replicates for each.

Cheese-making

The Italian cheese-making procedure was followed, with modifications, expressed by the use of the pasteurized milks (buffalo and cow) at 65ºC/30 minutes and the use of standardized buffalo milk at 4.1% fat content. After, the same Italian steps were followed.

Chemical composition and physical characteristics

Milk: The physical analysis as pH, titratable acidity (ºD) and density (g/mL), at 15ºC by Quevenne's termolactodensimeter was carried out in triplicate. We determined fat content by Gerber, total nitrogen (TN) by Kjeldahl, a correction factor of 6,38, for total protein determination, lactose by Fehling using the reduction method, total solids (TS) by gravimetric method, solids non fat (SNF) estimated by difference between TS and fat content and moisture, estimated by TS-100% (BRASIL, 2006).

Mozzarella cheese: Moisture was determined by gravimetric method at 105°C and ash content in muffle at 550°C. We determined fat content by Gerber method, total nitrogen (TN) by Kjeldahl, a correction factor of 6,38, for total protein determination, total solids by difference between moisture and solids, and pH by digital pH meter [18].

Instrumental analysis

Color: The color was determined by Color quest XE (Hunter Lab) of universal software, calibrated in black and white surface. CIE L*a*b was used [19], where: L* represent a luminosity on a scale from 0 (black) to 100 (white); a* chromaticity, scale from red (0+a) to green (0-a) and b* chromaticity that represents a scale from yellow (0+b) and blue (0-b).

Texture: Cheese texture was determined by TA HD plus (Stable Micro Systems Ltd., England), adjusted with 50 Kg cell charge, with cylindrical probe 100 mm (P100). The samples were collected with cylinder (22 mm × 26 mm, diameter) at 20ºC, disregarding the borders.

Double compression was used with parameters: pre-test velocity 1,0 mm/s, test velocity 2,0 mm/s, post-test velocity 2,0 mm/s, deformation rate 70%, with 5 seconds between 1º and 2º compression cycle. The data was analyzed by Texture Exponent Version 3.2 software. We used nine replicates per treatment. We analyzed the primary attributes of hardness, cohesiveness and elasticity, and the secondary attribute of chewiness.

Melt ability

The melt ability (MP) was tested in triplicate cheese disc (26 mm diameter and 7 mm depth) using adapted Schreiber's method for cheese. These discs were heated at 105ºC for 7 minutes, and the increase in disk diameter was measured at six points and averaged. A value of 1.0 indicated no change in disk diameter and melt values increased by 1.0 for every 1.0 cm increase in diameter [20].

Statistics

Milk and cheese composition, instrumental characteristics and melt ability data were analyzed by variance analysis (ANOVA) and the means compared by F-test (α=0,05) and regression analysis. These statistical models were chosen according to significance level and coefficients of determinations (R^2) by F-test. We used R software (R–Development Core Team, 2013).

Results and Discussion

Chemical composition and physical characteristics

The milk of the buffaloes presented higher fat, protein, lactose, TS, SNF and density ($P<0.05$) than cow's milk, except the moisture content (Table 1), as related by [21] that found higher TS in the buffalo's milk, correlating those results to more fat, protein and lactose content. The same behavior was observed by Ménard, Ahmad, Rousseau, Briard-Bion and Gaucheron [22] and Ahmad Gaucher, Rousseau, Beaucher, Piot, Grongnet and Gaucheron [23]. Milk components could be change by ambient factors such as: season and nutrition. Also, breed, age, lactation stage, sanitation, food, genetics polymorphism and specie [24-30].Those effects were not evaluated in this study.

The mozzarella from water buffalo's milk presents specific chemical composition; however, in Brazil there is no specific legislation, while in Italy, buffalo mozzarella is sold under the designation "Mozzarela di Bufala Campana" with PDO and should present specific characteristics [1-3]. Mozzarela from buffaloes and cows presented significant difference in the composition ($P<0,05$) for fat in dry matter (FDM), protein, humidity, TS and ash, with the exception of pH (Table 1), similar to what was related by Saanen et al. (2008).

The maximum moisture content for the "Mozzarella di Bufala Campana" should be 65% and fat on dry matter (FDM) 52% [1]; therefore, results observed in this study are in accordance with these recommendations. The treatments (0%, 10%, 20%, 30%, 40% and 50%) changed the FDM and protein content in the mozzarella (Table 2). The cow's milk addition produced quadratic effects on the FDM ($P<0,003$). Despite this behavior, the data showed, in absolute values, that the FDM

Variables	Milk		*P*-value	Mozzarela		*P*-value
	Buffalo	Cow		Buffalo	Cow	
pH	6.72 ± 0.06	6.69 ± 0.02	0.088	6.62 ± 0.04	6.48 ± 0.02	0.185
Fat (%)	4.26 ± 0.71	4.05 ± 0.04	0.000	60.57 ± 0.53*	57.32 ± 0.87*	0.025
Protein (%)	3.05 ± 0.21	2.92 ± 0.04	0.003	23.82 ± 0.69	20.87 ± 0.49	0.016
TS (%)	12.47 ± 0.29	12.02 ± 0.12	0.000	53.70 ± 0.21	51.56 ± 0.58	0.095
Moisture (%)	-	-	-	46.30 ± 0.44	48.44 ± 0.55	0.076
Ash (%)	-	-	-	2.89 ± 0.12	2.55 ± 0.21	0.040
Lactose (%)	4.27 ± 0.26	4.14 ± 0.05	0.003	-	-	-
SNF (%)	8.21 ± 0.41	7.97 ± 0.11	0.002	-	-	-
Titratable acidity (ºD)	15 ± 1.41	15 ± 1.31	0.071	-	-	-
Density (g/mL)	1.029 ± 0.01	1.028 ± 0.02	0.009	-	-	-

TS: Total Solids; SNF: Solids Non Fat, F-Test: P<0.05., *Fat In Dry Matter For Cheese

Table 1: Physical-chemical composition of the milk and mozzarela cheese from buffalo and cow (average ± standard deviation).

was lower when cow's milk was added. The buffalo's milk presented higher fat than cow's milk, a fact that can explain this behavior (Table 1).

This result can explain, using the coagulation process, variations in the amount of fat globules retained in the protein network. According to Walstra, Geurtz, Noomen, Jellema and Van Boekel [31], during coagulation, under proteolytics enzymes and/or lactic acid, changes are observed in casein micelles that can aggregate, forming a gel. This gel is formed by small linked particles (protein chain) that constrain the fat globules. These 40% and 50% cow's milk inclusion levels presented the lowest FDM, below 52% fixed by "Mozzarela di Bufala Campana." However, for the moisture content, all treatments are in accordance with that legislation [1]. The protein content was reduced with cow's milk inclusion, linearly (Table 2). The protein is one of the main milk components, linked with total solids. While the cow's milk addition has not affected the TS content, this addition produced cheeses with lower protein content, reducing 10.7% in the average.

Instrumental analyzer

Significant difference was observed between mozzarella from cows and buffaloes, in relation to L* luminosity and a* and b* chromaticity (Table 3). The β-caroten content, as well as hydration and mineralization, can influence the milk's color and consequently the cheese color [23,32]. So, the milk whiteness results, mainly, scattering light from the presence of colloidal particles as fat globules and casein micelles that can impact the free water [33,34].

The buffalo's mozzarella is characterized by whiteness, probably, from lower β-caroten, casein micelles' higher mineralization and lower hydration than cow's [23,32]. Indeed, it was expected that mozzarella from buffalo provides a greater value of L*, in relation to cheese from cows, which was not observed in this study. Higher L* value is determined for lower fat and protein content. When this happen, the TS is reduced, elevating the free water, resulting in lowest scattering light, tending to white [33,34]. Our results corroborate this affirmative. The mozzarella from cows presented a higher L* value than from buffalo, and in turn, presented lower fat and protein content (Table 1). Thus, cheeses with lower fat content present a lower number of the centers that permit scattering light, making them less opaque. Changes in opacity can also be related to the degree of internal aggregation of the cheese proteic matrix where the higher hydration determines the number of centers that allow the light to spread.

The mozzarella from buffalo presented negative a* values (-2.35), trending to green. Green precipitate is originated from the biliverdine, α-caseína associated pigment, precipitated by heat or acid action. Probably, the milk from buffalo presents more biliverdine content, which can explain the tendency to green color for the buffalo mozzarella. The b* value (20.01) observed for the mozzarella from cow, show the tendency to yellowness for this cheese, in relation to buffalo's mozzarella (15.47). Carotenoids, as in β-carotene (from the milk), determine yellowness in cheese. Thus, buffalo's milk is poor in β-carotene when compared to cow's milk; however, it is rich in vitamin A [32].

The buffalo's mozzarella present higher attributes for firmness, chewiness, elasticity and cohesiveness than cow's (Table 3). Firstly, the physical properties of the cheeses are influenced by milk composition, cheese-making and maturation [10]. In this study, all conditions are similar, except for the milk. The buffalo's mozzarella, normally, present an elastic and firm texture, and results show those characteristics. As observed, the buffalo's milk has higher fat, protein, and lactose content as well as TS and FDM. The milk composition can reflect in the cheese composition. Except for the moisture, all variables such as FDM, protein, ash and TS were higher in the mozzarella from buffaloes, while the pH was similar (Table 1). Probably, this response can be explained in function of the milk compositions (physical and chemical), which will influence the cheese's macro-structure, especially the Ca [23].

Cheese is a visco-elastic (gel) composed of a casein chain, fat globules and dispersed water in its interior [35], which exerts remarkable effects

Variables	Cow milk level (%)						VC (%)	*P-value*		
	0	10	20	30	40	50		L	Q	C
pH	6.62	6.58	6.80	6.77	6.76	6.86	2.98	0.104	0.093	0.078
FDM (%)[1]	60.57	53.93	52.18	53.93	51.28	51.43	3.61	0.122	0.003	0.097
Protein (%)[2]	23.82	22.32	22.25	20.84	20.80	20.20	4.48	0.000	0.383	0.809
Moisture (%)	46.30	45.57	46.38	48.12	46.61	47.48	3.56	0.206	0.885	0.527
Ash (%)	2.89	2.90	3.04	2.98	3.12	2.85	2.87	0.375	0.425	0.212
TS (%)	53.70	54.43	53.62	51.88	53.38	52.52	3.13	0.206	0.885	0.527

FDM: Fat In Dry Matter; TS: Total Solids; F-test: P<0.05;
Variation Coefficient Linear: L; Quadratic: Q; Cubic: C Effects
1 $\hat{Y}$=0.0067X2 - 0.4705X + 59.578 (R^2=0.86)
2 $\hat{Y}$=-0.0688X + 23.424 (R^2=0.92)

Table 2: Chemical composition and physical characteristics to the mozzarela from buffalo added with cow milk during cheese-making.

Color	Mozzarela		P-value
	Buffalo	Cow	
L*	88.47 ± 0.18	89.53 ± 0.13	0.001
a*	-2.35 ± 0.24	1.84 ± 0.11	0.000
b*	15.47 ± 0.61	20.01 ± 0.68	0.006
Texture			
Firmness (N)	85.17 ± 0.59	33.46 ± 0.45	0.005
Chewiness (N)	33.93 ± 0.65	8.99 ± 0.54	0.005
Elasticity	0.863 ± 0.01	0.702 ± 0.10	0.153
Cohesiviness	0.449 ± 0.02	0.348 ± 0.02	0.002

F-test: P<0.05

Table 3: CIE L*a*b* color system and textural profile analysis (TPA) for mozzarela cheeses from buffalo and cow (average ± standard deviation).

in the texture. Another factor also can influence texture characteristics, such as casein and the interactions of its molecules, the Ca with these, as well as the proteolysis. In turn, these are affected by environmental conditions, such as pH development, temperature and ionic linkages. The buffalo's mozzarella presented higher firmness and chewiness than cow (Table 3). In the curd, the protein exerts remarkable effects, as well as the water and fat. However, the moisture/protein rate exerted strong effects on the curd [35,36]. The mozzarella from buffalo presented lower moisture than cow's mozzarella, while protein content was higher (Table 1). The moisture/protein rates are different in both mozzarellas, with the highest water content in the cow's mozzarella.

Also, factors such as casein micelles can influence texture parameters [37]. The casein micelles from buffalo's mozzarella are larger than observed in the cow's mozzarella. This configuration determines the lowest water retention during curd-making, which may have influenced the cheese firmness [23,38]. Cheese with the lowest moisture can lean to lower cohesiveness. However, the buffalo's mozzarella presented larger cohesiveness, even having lower moisture. The protein, fat, lactose, TS and SNF were larger in the buffalo's milk (Table 1). Those differences probably changed the proteic matrix configuration, which may have influenced the cheese texture (Table 3). Was observed similarity in the acidification between buffalo and cow's milk; however, the quantification is different and may induce different processes [23].

The Ca content in the milk from buffalo is larger as well as the Ca associated with casein [23], indicating different acidification processes among species. Thus, those results observed in this study can be explained by differences observed in the chemical process during curd-making and milk composition. It is necessary to highlight the serina αs1-casein fosforilation, similar in both milks, except the serina 115, absent in buffalo's milk. This absent makes αs1-casein more hydrophobic [39].

The cow's milk included in the buffalo's milk altered the L* value of the mozzarella (Table 4). As said, L* is the parameter that can evaluate the ability of the object to reflect light; the closer to 100, the more whiteness. This observation confirms the difference between buffalo and cow's mozzarella, as related in Table 3. At the same, higher L* value (Table 4) can be related with protein and FDM content observed in those samples (Table 2). With the addition of the cow's milk in buffalo's milk a* and b* chromaticity were altered, linearly and positively ($P<0.05$) (Table 4). This result can be correlated with previous observations that compared mozzarella cheese from buffalo and cows (Table 3). The yellowness was also observed visually to the extent that it included the cow's milk, in function of the β-carotene, a cow characteristic. The cheese color can be influenced by fat content. Higher fat content was observed in the mozzarella from buffalo (Table 1), which showed results with a* negative values (Table 4). When cow's milk was added into the buffalo, we observed an increase in a* value, and reduction in the GES (Table 2).

A linear decreasing effect was observed ($P<0.05$) for firmness, chewiness, elasticity and cohesiveness to the extent that cow's milk was added to buffalo's milk (Table 4). The buffalo's mozzarella is characterized by firmness and elasticity. Those attributes are affected with cow's milk inclusion, producing the lowest firmness and elasticity, characterized as less firm samples, with lowest tendency to return the original form after withdrawal force. The cow's milk inclusion, also decreasing the cohesiveness (sample deformation degree before the rupture), reducing the chewiness. Indeed this is expected, because the chewiness is correlated to elasticity.

The cow's milk inclusion in the buffalo's milk affected the protein content in the cheeses (Table 1). This resulted in a decrease of the elasticity and firmness in the cheeses, most likely due to a less compact curd structure (proteic matrix) in the cow's mozzarella, which has determined the lower number of the fat globules retained the proteic matrix, which influenced the parameters of the texture. The casein micelles are largest in the buffalo's cheese [40-42]. Thus, the bridges between casein and molecules near to them are quickly strengthened, avoiding rearrangements in curd, which determines greater softness and firmness for buffalo's curd. The identification of the species from which the milk/cheese are sourced has importance to the traceability of food and fraud control [3]. Thus, instrumental tests showed capacity for the use as a screening test for identifying imitations in the buffalo's mozzarella with cow's milk, presenting itself as potential new alternative to detect frauds.

Melt ability

The melt ability is associated with phase change that occurs when the cheese is heating, when that fat changed from solid phase to liquid, in function to heat. The cow's milk inclusion into the buffalo's milk produced quadratic effect ($P<0.05$) in the cheese melt ability. The cow's milk addition (10%) induced decreasing in the melt ability, when was added 20% of cow`s milk the melt ability was increasing. The other treatments decreased the melt ability. This behavior can be explained

Color	Cow milk levels (%)						P-value		
	0	10	20	30	40	50	L	Q	C
L*[1]	88.47	89.94	90.36	90.07	90.72	90.33	0.266	0.040	0.295
a*[2]	-2.35	-1.78	-1.67	-1.00	-0.64	-0.24	0.000	0.911	0.944
b*[3]	15.47	17.28	18.52	18.75	19.01	19.34	0.022	0.286	0.338
Texture									
Firmness (N)[4]	85.17	83.56	80.26	74.76	67.47	51.27	0.001	0.839	0.823
Chewiness (N)[5]	33.93	32.47	31.75	23.18	22.61	18.32	0.003	0.466	0.557
Elasticity[6]	0.863	0.804	0.800	0.777	0.774	0.761	0.003	0.474	0.508
Cohesivity[7]	0.449	0.413	0.408	0.400	0.392	0.371	0.022	0.303	0.592

F-test: $P<0.05$
Linear: L; Quadrátic: Q; Cúbic: C Effects
1 $\hat{Y}=-0.0013X2+0.0897X+88.779$ ($R^2=0.67$)
2 $\hat{Y}=0.0418X-2.3257$ ($R^2=0.98$)
3 $\hat{Y}=0.0706X+16.293$ ($R^2=0.83$)
4 $\hat{Y}=-0.6379X+89.696$ ($R^2=0.87$)
5 $\hat{Y}=-0.332X+35.341$ ($R^2=0.92$)
6 $\hat{Y}=-0.0018X+0.841$ ($R^2=0.92$)
7 $\hat{Y}=-0.0013X+0.4385$ ($R^2=0.92$)

Table 4: CIE L*a*b* color system and textural profile analysis (TPA) for mozzarela cheese from buffalo milk with inclusion of the cow milk.

by decreasing the FDM content (Table 1) in addition to decreasing the firmness (Table 4) with the increase in the inclusion of cow's milk, which had trouble breaking the protein matrix during the heating. The melt ability is a purposeful technological property that determines the quality of the product. Cheeses are used in foods, especially the mozzarella, used in pizzas, should present a uniform melting without large bubbles formation. Thus, the buffalo's mozzarella showed great melt ability, since uniformity and the absence of bubbles were observed.

Conclusion

The measures of instrumental color, texture and melt ability indicated that the cheese made with different levels of cow's milk presented distinct characteristics of the mozzarella from buffalo milk alone. Instrumental tests such as color, texture and melt ability can be used in the detection of the buffalo's mozzarella adulteration by cow's milk, showing a potential alternative for the quality control of the buffalo's mozzarella cheese.

Acknowledgement

Thanks to CAPES, for the grant and to Rocha and Pitty dairies for collaboration with this study.

References

1. DM (2003) Decreto del Ministero delle Politiche Agricole e Forestali del 18 settembre 2003. Disciplinare di produzione della denominazione di origine protetta della "Mozzarella di Bufala Campana". Gazzetta Ufficiale N.258 del 6 novembre.
2. European Union (EU) (1996) Commission Regulation (EC) No. 1107/96 of 12 June 1996 on the registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No. 2081/92. Official Journal of the European Commission 1-15.
3. Dalmasso A, Civera T, Neve F, Bottero MT (2011) Simultaneous detection of cow and buffalo milk in mozzarella cheese by Real-Time PCR assay. Food Chemistry 12: 362-366.
4. ABCB (Associação Brasileira de Ciradores de Búfalos) (2008) Associação brasileira de criadores de búfalos intensifica campanha por pureza de derivados do leite. Portal do Agronegócio.
5. Zicarelli L (1997) Reproductive seasonality in buffalo. Bubalus bubalis 29-52.
6. Locci F, Ghiglietti R, Francolino S, Iezzi R, Oliviero V, et al. (2008) Detection of cow milk in cooked buffalo mozzarella used as pizza topping. Food Chemistry 107: 1337-1341.
7. Czerwenka C, Miller L, Lindner W (2010) Detection of the adulteration of water buffalo milk and mozzarella with cow's milk by liquid chromatography–mass spectrometry analysis of β-lactoglobulin variants. Food Chemistry 12: 901-908
8. Foegeding EA (2007) Rheology and sensory texture of biopolymer gels. Current Opinion in Colloid & Interface Science 1: 242-250.
9. Foegeding EA, Drake MA (2007) Invited review: Sensory and mechanical properties of cheese texture. J Dairy Sci 90: 1611-1624.
10. Lucey JA, Johnson ME, Horne DS (2003) Invited review: perspectives on the basis of the rheology and texture properties of cheese. J Dairy Sci 86: 2725-2743.
11. Lassoued N, Delarue J, Launay B, Michon C (2008) Baked product texture: correlations between instrumental and sensory characterization using Flash Profile. Journal of Cereal Science 48: 133-143.
12. Addeo F, Garro G, Intorcia N, Pellegrino L, Resmini P, et al. (1995) Gel electrophoresis and immunoblotting for the detection of casein proteolysis in cheese. J Dairy Res 62: 297-309.
13. Russo R, Severino V, Mendez A, Lliberia J, Parente A, et al. (2012) Detection of buffalo mozzarella adulteration by an ultra-high performance liquid chromatography tandem mass spectrometry methodology. J Mass Spectrom 47: 1407-1414.
14. Commission Regulation (2001) Commission Regulation No. 213/01 of 9 January, 2001 lying down detailed rules for the application of Council Regulation (E.C.) No. 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products and amending Regulations (E.C.) No. 2771/1999 and (E.C.) No. 2799/1999. Official Journal of the European Communities.
15. Bottero MT, Civera T, Anastasio A, Turi RM, Rosati S (2002) Identification of cow's milk in "buffalo" cheese by duplex polymerase chain reaction. J Food Prot 65: 362-366.
16. Li R, Carpenter JA, Cheney R (1998) Sensory and instrumental properties of smoked sausage made with Mechanically Separated Poultry (MSP) meat and wheat protein. Journal of Food Science 6: 5.
17. Shoemaker CF, Nantz J, Bonnans S, Noble AC (1992) Rheological characterization of dairy products. Food Technology 46: 98-104.
18. Brasil (2006) Instrução Normativa nº 68, de 12 de dezembro de 2006. Oficializa os Métodos Analíticos Oficiais Físico-Químicos para Controle de Leite e Produtos Lácteos. Diário Oficial da União, Poder Executivo, Brasília, DF, 14 dez. Seção 1. Brazilian Official Journal.
19. Commission Internationale de l'Éclairage (CIE) (1996) Colorimetry. (2nd edition) CIE publication, Vienna.
20. Kindstedt F, Mistry VV (1997) Cheese and fermented milk foods. Edwards Brothers, Michigan 323.
21. Mahmood A, Usman S (2010) A comparative study on the physicochemical parameters of milk samples collected from buffalo, cow, goat and sheep of gujrat. Pakistan Journal of Nutrition 9: 1192-1197.
22. Ménard O, Ahmad S, Rousseau F, Briard-Bion V, Gaucheron F, et al. (2010) Buffalo vs. cow milk fat globules: Size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. Food Chemistry 120: 544-551.
23. Ahmad S, Gaucher I, Rousseau F, Beaucher E, Piot M, et al. (2008) Effects of acidification on physicochemical characteristics of buffalo milk: A comparison with cow's milk. Food Chemistry 106: 11-17.
24. Talpur FN, Memon NN, Bhanger MI (2007) Comparison of Fatty Acid and Cholesterol Content of Pakistani Water Buffalo Breeds. Pakistan Journal of Analytical and Environmental Chemistry 8: 15-20.
25. Bhonsie D, Chourasia SK, Singh M, Jain RK (2003) Factors influencing major milk constituents in Murrah buffaloes. Indian Journal Animal Science 7: 107-109.
26. Sodi SS, Mehra ML, Jain AK, Trehan PK (2008) Effect of non-genetic factors on the composition of milk of Murrah buffaloes. Indian Veterinary Journal 8: 950-952.
27. Tufarelli V, Dario M, Laudadio V (2008) Diet composition and milk characteristics of Mediterranean water buffaloes reared in South Eastern Italy during spring season. Livestock Research for Rural Development 20: 1-7.
28. Nawaz H, Yaqoob M, Sarwar M, Abdulla M, Sultan JI, et al. (2009) Effect of feeding different sources of supplemental fat on the performance of Nili-Ravi buffaloes. Indian Journal Animal Science 79: 188-192.
29. Rajala-Schultz PJ, Gröhn YT, McCulloch CE, Guard CL (1999) Effects of clinical mastitis on milk yield in dairy cows. J Dairy Sci 82: 1213-1220.
30. Misra SS, Sharma A, Bhattacharya TK, Kumar P, Saha RS (2008) Association of breed and polymorphism of ?-s1and ?-s2casein genes with milk quality and daily milk and constituent yield traits of buffaloes (*Bubalus bubalis*). Buffalo Bulletin 27: 294-301.
31. Walstra P (2001) Ciencia de la leche y tecnología de los productos lácteos. (1st ed) Zaragoza: Acribia, Spain.
32. Bergamo P, Fedele E, Lannibelli L, Marzillo G (2003) Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. Food Chemistry 8: 625-631.
33. Fennema OR (1993) Química de los alimentos. (2nd ed) Zaragoza: Acribia, Spain pp: 1095.
34. García-Pérez FJ, Lario Y, Fernández-López J, Sayas E, Pérez-Alvarez, et al. (2005) Effect of orange fiber addition on yogurt color during fermentation and cold storage. Color Research & Application 30: 457-463.
35. Prentice JE (1987) Cheese rheology. In: Cheese: Chemistry, Physics, and Microbiology. Elsevier Applied Science, New York pp: 299-344.

36. Walstra P, Noomen A, Guerts TJ (1987) Cheese: Chemistry, Physics and Microbiology. (2nd ed) Elsevier applied science publishers, London.

37. Patel HG, Upadhyay KG, Miyani RV, Pandya AJ (1993) Instron texture proflle of buffalo mllk cheddar cheese as influenced by composition and ripening changes. Food Quality and Preference 4: 187-192.

38. Ganguli NC (1979) Tecnología de la leche de búfala. Revista Mundial de Zootecnia 30: 2-10.

39. Ferranti P, Scaloni A, Caira S, Chianese L, Malorni A, et al. (1998) The primary structure of water buffalo α s1- and β -casein: identification of phosphorylation sites and characterization of a novel β - casein variant. Journal of Protein Chemistry 17: 835-844.

40. Hussain I, Bell AE, Grandison AS2 (2011) Comparison of the rheology of mozzarella-type curd made from buffalo and cows' milk. Food Chem 128: 500-504.

41. Dean CB, Nielsen JD (2007) Generalized linear mixed models: a review and some extensions. Lifetime Data Anal 13: 497-512.

42. Sameen A, Anjum FM, Huma N, Nawaz H (2008) Quality evaluation of mozzarella cheese from different milk sources. Pakistan Journal of Nutrition 7: 753-756.