EVALUATION OF THE EXPERIMENTAL FRAUD OF BUFFALO CHEESE BY SENSORY ANALYSIS AND PHYSICO-CHEMICAL PARAMETERS

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ABSTRACT

The objective of the present study was to identify the physico-chemical differences between experimentally fraudulent cheeses and those produced exclusively with cow and buffalo milk and to sensorially assess the consumer's perception of these products. Three types of cheese were produced using buffalo milk and cow milk as raw materials at different proportions, and total lipids were analysed; protein ash, moisture and carbohydrate contents were measured; energy values were determined; and affective sensory and purchase intent analyses were performed. The results indicated that 57% and 59% of the judges, respectively, reported liking the colour and texture of the mixed cheese very much and that they would certainly buy it. Regarding the composition, the fraudulent cheese had a lower energy value compared to the cow and buffalo cheeses and that the protein content of the fraudulent cheese was lower than that of the buffalo cheese. There were no significant differences in the carbohydrate or fat contents among the analysed products. Compared with the cow cheese, the mixed cheese and buffalo

cheese had higher moisture contents. It was concluded that although the mixed cheese presented significant physico-chemical differences, it was considered acceptable product because consumers showed intent to purchase the mixed cheese.

Keywords: *Bubalus bubalis*, consumer, buffalo, cheese.

INTRODUCTION

The buffalo (*Bubalus bubalis*) is an animal originally from the tropical and temperate zones of the Asian continent and is well-adapted to these climatic conditions (Bastianetto, 2009). The great advantages of buffalo farming may be attributed to their docile temperament and large digestive capacity and the triple use of these animals because, although they are traditionally farmed for meat production, they are an important source of high-quality milk and can be used as traction animals (MEI *et al.*, 2008). Due to these advantages and their high availability, several industrial facilities produce a variety of buffalo

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milk derivatives, especially cheese, and they opt to use this raw material due to the superiority of its components compared to cow milk (Bittencourt et al., 2013). Despite the growth of buffalo dairy farming, the consumption of food products from this production chain is informal due to the lack of regulation of standards for the identity and quality of buffalo milk and its derivatives, which complicates the control and inspection measures (Pereira Júnior et al., 2009). This fact is favourable for the incidence of fraud, especially through the addition of cow products to products that should be produced exclusively with buffalo raw materials. The Regulation of Industrial and Health Inspection of Products of Animal Origin (Regulamento da Inspeção Industrial e Sanitária dos Produtos de Origem Animal, RIISPOA) (Brasil, 1956) describes fraud as "the total or partial alteration or modification of one or more typical product elements during handling and preparation, with the deliberate intention of establishing a false impression of the manufactured products when one or more elements are suppressed and substituted by others or when there is total or partial specification on the label of a certain product that is not contained in the package or container". Therefore, any incorporation or substitution of a product component that is not described on the label is considered an irregularity, which has been reported in buffalo cheeses (Silva et al., 2015). Many studies have been conducted to detect fraud in cheese samples (Dalmasso et al., 2011; Golinelli et al., 2014; Guerreiro et al., 2012; Lopparelli et al., 2007; López-Calleja et al., 2005; Mininni et al., 2009; Motta et al., 2014; Sachinandan et al., 2011; Teixeira et al., 2012; Zhang et al., 2007). Silva et al. (2015) evaluated cheese samples sold as being of buffalo origin in municipalities from North Brazil and detected fraud by the incorporation of cow

milk, thus demonstrating the occurrence of this adulteration in this part of the country.

However, although this practice is illegal, there are no reports showing that incorporation of cow milk affects the sensory quality of the product, and currently, there are no scientific studies demonstrating whether the production of a mixed cheese, containing both buffalo and cow milk, would be accepted by the consumer. For such a study, methods such as sensory analysis can be useful tool given that it could provide evidence to the industry or even the producer as to whether there is a consumer market for mixed products, and in the case that there is, it could favour the formulation of a new product, thus discouraging fraud

Sensory analysis has been applied by different researchers to analyse the consumer market of products made from buffalo raw materials (Marques *et al.*, 2015). However, studies that apply it with the objective of testing the acceptance of fraudulent products are still insipient.

Furthermore, fraud involving the incorporation of milk of another species into cheese can alter the nutritional characteristics of the product, and thus, knowledge of the proximate composition of these dairy products is essential for determining the quality of the product. In addition to defining several organoleptic and industrial properties, this type of analysis also clarifies for the consumer the nutritional value of the product being purchased.

The objective of the present study was to evaluate whether the experimental fraud of buffalo cheeses through the addition of cow milk significantly alters the physico-chemical parameters of the product and to identify the sensory acceptance of this product by consumers.

MATERIALS AND METHODS

For the accomplishment of the present study three cheese batches were produced, each composed of 15 units of fresh cheese weighing approximately 250 g. The cheeses were prepared using buffalo milk and cow milk as raw materials at the proportions of 100% cow milk (C1), 50% buffalo milk and 50% cow milk (C2) and 100% buffalo milk (C3). Thus, each batch contained 5 units each of 3 types of cheese, which were used for the sensory analyses as well as the physicochemical tests.

For the sensory analysis, untrained judges (minimum of 30 and maximum of 60 judges) were recruited randomly among graduate and postgraduate students of a public university in the state of Pará, according to their willingness to participate in the study and the recommendations of the Adolfo Lutz Institute (IAL, 2008). which suggests that the health conditions of the participants, including the absence of colds, allergies, diabetes, hypercholesterolemia, and other conditions, be considered. The participants were classified according to their income, following the recommendations of the Brazilian Strategic Affairs Secretariat (Secretaria de Assuntos Estratégicos, SAE), into 8 groups (extremely poor; poor but not extremely poor; vulnerable; lower middle class; middle class; upper middle class; lower upper class and upper class).

The age range of the judges included in the study was 18 to 50 years, considering that beyond this range the individual may present some desensitisation of their sensory organs. All participants signed an informed consent form, which ensured that all measures were taken to guarantee their integrity and concealment of data that could identify them as well as data privacy and confidentiality.

The cheeses were then labelled with three-digit numeric codes and randomly served to the judges in 3 cm x 3 cm x 0.5 cm portions at refrigeration temperature. The tasters were instructed to drink one glass of water between consuming the samples and to mark their evaluation and comments in a survey that contained the following four sections: 1-Socioeconomic data; 2-Acceptance test using a five-point hedonic scale ranging from "liked very much" to "disliked very much" for colour, flavour and texture; 3-Purchase intent test using a 4-point hedonic scale ranging from "certainly would buy" to "certainly would not buy"; and 4-Order of preference test of the samples, performed according to the recommendation by Meilgaard, Civille and Carr (1991). To perform the proximate composition analysis, a pooled sample of each type of cheese was prepared, by batch, and the analysis was performed in triplicate with each replicate thus representing one batch. The following methods were employed: the Goldfisch method for the quantification of total lipids; calcination in a muffle furnace at 550°C for the determination of ash (IAL, 2008); the Kjeldahl method for protein content; and oven drying at 105°C to a constant weight for the determination of moisture and total volatiles (AOAC, 2000). In turn, the carbohydrate content and the energy value were determined indirectly, following the recommendation of Brasil (1996). Once the results were obtained, the analysed cheeses were classified according to their moisture content as having low, average, high and very high moisture content and based on their lipid content as being extra fat, fat, semi-fat, non-fat and skimmed, as described in the Technical Regulation of Identity and Quality of Cheeses (Brasil, 1996). The data from the analysis of the percent composition, energy value, carbohydrates, sensory analysis for acceptance, and purchase intention using a hedonic scale were analysed using analysis of variance (ANOVA), and in the case of a significance difference, Tukey's test was applied at a 5% significant level. In turn, the results obtained from the order of preference were subjected to the Friedman test at a 1% significance level. All statistical tests were performed in BioEstat version 5.3.

RESULTS AND DISCUSSION

The sensory analysis of food products is an important tool, as it provides critical information for their production and sale in terms of consumer preferences and demands (Iannario et al., 2012). In the present study the sensory analysis was performed by a group of untrained tasters from the academic community of a public university, and the data acquired indicated that the group was formed by 51 individuals, of which 67% (34/51) were women and 33% (17/51) were men. Of the tasters that participated in the study, 82% (42/51) were aged between 18 and 25 years, and all declared that they had completed secondary education and were enrolled in graduate or post-graduate courses. In regards to the socioeconomic evaluation, 6% (3/51) of participants reported having household income below 1 minimum salary (MS), 24% (12/51) between 1 and 2 MS, 47.1% (24/51) between 2 and 5 MS, and 22% (11/51) between 5 and 13 MS; only 2% (1/51) declared income higher than 13 MS. These data indicate that most study participants belonged to the middle class according to the classification by the SAE. Were estimated mean values indicated by the study participants in the hedonic scale for the sensorial attributes and purchase intent of fresh cheese produced with different percentages

of buffalo milk and cow milk (Table 1). In relation to the evaluated cheeses, no significant differences (P<0.0001) were observed among them in terms of their colour and texture according to the opinion of tasters. In regards to the same attributes, the mixed cheese (C2) had good acceptance, given that 57% (29/51) of judges claimed to like its colour very much and 59% (30/51) to like its texture very much. In relation to flavour, there was a significant difference between the cow cheese (C1) and buffalo cheese (C3). However, in regards to the mixed cheese (C2), no significant differences in flavour were observed when it was compared to the cow cheese or buffalo cheese.

The results of the sensory analyses suggest that the experimentally produced mixed cheeses could be consumed without being identified by the palate of possible buyers, and the same is true for commercially available fraudulent cheeses. Additionally, the data suggest the possible existence of a consumer market for buffalo cheese containing cow milk and that this cheese is accepted as much as the cow and buffalo cheeses, which could be explained by the organoleptic similarity between the products of cow and buffalo origin. The similarity between the products of buffalo and cow origin has been reported by many studies. Some studies have observed that the consumer cannot distinguish between cattle meat and buffalo meat due to the absence of significant differences between them in characteristics such as odour, flavour, tenderness and juiciness, and most consumers state that they like buffalo meat very much and cattle meat moderately. Therefore, buffalo meat is a potential substitute for cattle meat (Marques, et al., 2015; Ohly, 1997). This situation may have occurred in the present study as well, which explains the non-identification of the different cheeses by the tasters. In regards to

the purchase intent for the produced cheeses, no significant differences (P<0.0001) were observed, which indicated that the study participants would purchase the three samples. This finding corroborates the overall evaluation from the order of preference test, which indicated the absence of significant differences (P<0.0001) among the samples from the tasters' perspective. Although there are organoleptic similarities between buffalo milk and cow milk, many studies show that buffalo milk offers relevant nutritional advantages when either consumed fresh or as derivatives, especially cheese (El-Salam and El-Shibiny, 2011; Oliveira, et al., 2009; Pignata et al., 2014). However, Simões et al. (2013) note that the addition of up to 40% of cow milk in the production of buffalo cheese does not change the final quality of this product.

The means recorded for the cheese composition, carbohydrate content, energy values and classification in terms of the fat and moisture contents are shown in Table 2. It was observed that the protein content of the mixed cheese sample (C2) was lower than those of the buffalo (C3) cheese; the mixed cheese (C2) also had a lower energy value than those of the cow (C1) and buffalo (C3) cheeses. The moisture content was higher for the mixed cheese (C2) and buffalo (C3) cheese than for the cow (C1) cheese.

The high moisture contents found in the fraudulent and buffalo cheeses produced are most likely due to the large amount of denatured whey protein, which tends to increase the water retention capacity of these products. The moisture increase in the fraudulent cheeses analysed could be a favourable factor for a higher risk of contamination due to the increase in water activity, which favours the growth of pathogenic microorganisms. This factor should be considered, given that the fraudulent cheeses could be more susceptible

to microbial contamination at the conditions established in this study.

The fraudulent cheese had a lower protein content compared to the buffalo cheese. This result corroborates with the previous determination by Teixeira *et al.* (2005), who found that buffalo milk, used as a raw material for cheese, contains 48% more protein than cow milk. Although the three cheeses analysed could be considered important sources of protein, this result shows that by purchasing a fraudulent product, the consumer is also nutritionally damaged.

Additionally, casein is the most important protein in milk, and it represents 85% of dairy proteins, it forms micelles during the coagulation of fermented dairy products, and it has been found that the casein micelles in buffalo milk are larger than those found in cow milk, causing the curd prepared with buffalo milk to retain less water than that from cow milk, forming a more consistent product (FAO, 1991). This is a relevant aspect, given that mixed cheeses could have a different consistency due to the variation in the protein content, and this could influence the acceptance of a possible commercial product.

In regards to the carbohydrate and fat contents, there were no significant differences among the analysed products, and all cheeses were classified as low-fat according to the Technical Regulation of Identity and Quality of Cheeses (Brasil, 1996). Furthermore, in relation to ash content, there were no significant differences between the mixed cheese (C2) and buffalo cheese (C3); however, these products differed from the cow cheese (C1).

Silva and Ferreira (2010) evaluated the nutritional label, chemical composition and energy value of commercial cheeses and emphasised that the low carbohydrate contents found in some

Table 1. Mean values indicated by the study participants in the hedonic scale for the sensorial attributes and purchase intent of fresh cheese produced with different percentages of buffalo milk and cow milk.

Sample	Colour	Flavour	Texture	Purchase intent
C1	4.2a	4.3b	4.1a	4.0a
C2	4.4ª	3.9a	4.4ª	4.0a
C3	4.2ª	3.7ª	4.3ª	4.0ª

¹Means followed by the same letters in the same column indicate the absence of a significant difference at a 1% probability level; C1-cheese produced exclusively with cow milk; C2-cheese produced with 50% cow milk and 50% buffalo milk; C3-cheese produced exclusively with buffalo milk.

Table 2. Means and standard deviations for the energy value, percent composition and classification of fresh cheese produced with 100% cow milk (C1), 50% buffalo milk and 50% cow milk (C2) and 100% buffalo milk (C3).

Variables	C 1	C2	С3	Mean ±SD ³
Energy value	221.8±35.7a	169.8±39.0 ^b	258.9±13.4 ^{a.b}	216.8±48
Moisture	49±0.1a	65±0.6 ^b	56±0.8 ^b	57±6.71
Ashes	1.6±0.2ª	2.2±0.2 ^b	2.4±0.1 ^b	2.1±0.4
Fat	18±2.6a	15±0.5a	15±10.3a	16±6.3
Proteins	14±2.1a	13±1.3ª	20±0.2 ^b	16±3.6
Carbohydrates	9±0.6ª	4±2.2ª	13±9.9ª	8.8±6.9
Classification ² (Moisture/fat)	HM/LF	VHM/LF	VHM/LF	

¹Means followed by the same letters in the same row indicate the absence of a significant difference at a 1% probability level

^{*}Mean of the scores attributed to the cheeses by the judges.

²VHM: very high moisture; HM: high moisture; LF: low-fat.

 $^{^{3}}$ Mean \pm standard deviation for samples C1, C2 and C3.

of the analysed samples could be an indication of adulteration of the raw material used in the production of these samples. However, in the present study, although the mixed cheese contained a lower carbohydrate content, the difference was not significant when compared to the other cheeses.

Although the results are extremely relevant, further studies on the quantification of cattle DNA in commercial cheese samples should be performed to quantify the proportion of cow milk added to samples of fraudulent buffalo cheeses, considering that the nutritional loss of these cheeses is proportional to the amount of cow milk added, and for this purpose, the use of real time PCR proposed by many studies could be a viable alternative.

CONCLUSION

The partial substitution of buffalo milk with cow milk in the production of mixed cheese promoted some significant changes in the composition of this product. However, the sensory evaluations indicated that these differences were not clearly perceptible by the consumers that participated in the study, which demonstrates that the addition of cow milk in the production of buffalo cheese could lead to economic and nutritional losses by the consumer but that the production of mixed cheese could be an interesting market alternative given that it was well accepted.

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REFERENCES

Association of Official Analytical Chemists, AOAC. 2000. *Official 17: Methods of Analysis*. Washington, USA.Choudhary, P.C., B. Prasad and S.K. Misra. 1981. Note on the use of rumen liquor in the treatment of chronic alkaline indigestion in cows. *Indian J. Anim. Sci.*, **51**: 356-360.

Bastianetto, E. Criação de búfalos no Brasil: Situação e perspectiva. 2009. *Revista Brasileira de Reprodução Animal*, **6**: 98103.

Bittencourt, R.H.F.P.M., M.A.S. Cortez, E.T. Mársico, M.S.S.R. Rosa, C.M.A.D. Taxi and C.F.P.A.N. Ermita. 2013. Caracterização de Requeijão Marajoara e Minas Frescal produzidos com leite de búfalas no Estado do Pará, Brasil. *Cienc. Rural.*, **43**(9): 1687-1692.

Dalmasso, A., T. Civera, F. La Neve and M.T. Bottero. 2011. Simultaneous detection of cow and buffalo milk in mozzarella cheese by Real-Time PCR assay. *Food Chem.*, **124**: 362-366.

El-Salam, M.H.A. and S. El-Shibiny. 2011. A comprehensive review on the composition and properties of buffalo milk. *Dairy Sci. Technol.*, **91**: 663-699.

- Golinelli, L.P., R.S. Carvalho, C.S.C. Casaes, C.S.C. Lopes, R. Deliza and V.M.F. Paschoalin. 2014. Sensory analysis and species-specific PCR detect bovine milk adulteration of frescal (fresh) goat cheese. *J. Dairy Sci.*, **97**(11): 6693-6699.
- Guerreiro, J.S., P. Fernandes and R.G. Bardskey. 2012. Identification of the species of origin of milk in cheeses by multivariate statistical analysis of polymerase chain reaction electrophoretic patterns. *Int. Dairy J.*, **25**: 42-45.
- FAO. Food and Agriculture Organization. *The Buffalo*. Food and Agriculture Organization of the United Nations. **4**: 320.
- Iannario, M., M. Manisera, D. Piccolo and P. Zuccolotto. Sensory analysis in the food industry as a tool for marketing decisions. 2012. Adv. Data Anal. Classif., 6(1): 303-321.
- López-Calleja, I., A. Gonzalez, V. Farjado, M.A. Rodríguez, P.E. Hernández and R.M. García. 2005. PCR detection of cows' milk in water buffalo milk and mozzarella cheese. *Int. Dairy J.*, **15**: 1122-1129.
- Lopparelli, R.M., B. Cardazzo, S. Balzan, V. Giaccone and E. Novelli. 2007. Real-Time TaqMan polymerase chain reaction detection and quantification of cow DNA in pure Water buffalo mozzarella cheese: Method validation and its application on commercial samples. *J. Agric. Food. Chem.*, **55**: 3429-3434.
- Marques, C.S.S., R.P. Oaigen, C.M. Moraes, M.A.S. Santos, J.B.L. Júnior and I. Abel. 2015. Perfil dos consumidores da carne de búfalo, em Belém, Pará, Brasil. *Acta Veterinaria Brasilica*, **9**(2): 126-133.

- Mei, G.D., L. Hao, M.Q. Zhang, Z.C. Liang and L.Z. Ai. 2008. Production performance measurement for Dehong buffaloes. *China Animal Husbandry and Veterinary Medicine*, **35**: 136-138.
- Meilgaard, M., G.V. Civille and B.T. Carr. 1991.

 Sensory Evaluation Techniques. Apple
 Academic Press Inc., Oakville, Canada.
 354p.
- Mininni, A.N., C. Pellizzari, B. Cardazzo, L. Carraro, S. Balzan and E. Novelli. 2009. Evaluation of real-time PCR assays for detection and quantification of fraudulent addition of bovine milk to caprine and ovine milk for cheese manufacture. *Int. Dairy J.*, 19: 617-623.
- Ministério da Agricultura Pecuária e Abastecimento. 2016. *TIQQ Diário Oficial* [da] Republica Federativa do Brasil, Brasilia. Diário Oficial [da] União. Brasília, DF, Brasil.
- Motta, T.M.C., R.B. Hoff and F. Barreto. 2014.

 Detection and confirmation of milk adulteration with cheese whey using proteomic-likes ample preparation and liquid chromatography-electrospraytandem mass spectrometry analysis.

 Talanta, 120: 498-505.
- Ohly, J.J. 1997. Prova organoléptica com carnes bubalinas e bovinas de animais criados nas pastagens de várzeas da Amazônia central. *Acta Amazon.*, **27**(1): 33-42.
- Oliveira, R.L., M.M. Ladeira, M.A.A.F. Barbosa, M. Matsushita, G.T. Santos and A.R. Bagaldo. 2009. Composição química e perfil de ácidos graxos do leite e muçarela de búfalas alimentadas com diferentes fontes de lipídeos. *The Journal Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **61**(3):

- 736-744.
- Júnior, J.B.P., K.G. Fernandes and R.C.S. Muller. 2009. Determinação direta de ca, mg, mn e zn em amostras de leite de búfala da ilha de Marajó por espectrometria de absorção atômica com chama (faas). *Quim. Nova.*, 32(9): 2333-2335.
- Pignata, M.C., S.A.A. Fernandes, S.P.B. Ferrão, A.S. Faleiro and D.G. Conceição. 2014. Estudo comparativo da composição química, ácidos graxos e colesterol de leites de búfala e vaca. *Rev. Caatinga*, **27**(4): 226-233.
- Sachinandan, D., B. Brahma, S. Polley, A. Mukherjee, D. Banerjee, M. Gohaina, K.P. Singh, R. Singh and T.K. Datta. 2011.
 Simplex and duplex PCR assays for species specific identification of cattle and buffalo milk and cheese. *Food Control*, 22: 690-696.
- Silva, C.L., G.A. Sales, J.G.S. Neto, J.S. Silva, A.P.S.S. De Lara, C.G. Lima, F.P.L. Leite, E.S.C.L. Nunes, C.C.G. Moraes, T.B. Roos and C.M. Moraes. 2015. Detecção de fraude em amostras comerciais de queijo bubalino por adição de leite bovino por meio da técnica de Reação em Cadeia da Polimerase (PCR) multiplex. *Revista do Instituto Adolfo Lutz*, 74(1): 21-29.
- Silva, L.F.M. and K.S. Ferreira. 2010. Avaliação de rotulagem nutricional, composição química e valor energético de queijo minas frescal, queijo minas frescal "light" e ricota. *Alimentos e Nutrição Araraquara*, **21**(3): 437-441.
- Simões, M.G., A.F.N. Domingues, L.P. Maciel, J.G. Rabelo, E.B. Oliveira and C.L.L.F. Ferreira. 2013. Efeito da adição de leite bovino ao leite de búfala nas diferentes características

- do queijo artesanal do Marajó, tipo creme. *Revista do Instituto de Laticínios Cândido Tostes*, **68**(391): 32-40.
- Teixeira, L.V., E. Bastianetto and D.A.A. Oliveira. 2005. Leite de búfala na indústria de produtos lácteos. *Revista Brasileira de Reprodução Animal*, **29**(2): 96-100.
- Teixeira, L.V., C.S. Teixeira and E. Caldeira. 2012. Extração de DNA e avaliação da composição espécie-específica de queijos. *The Journal Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **64**(3): 721-726.
- Zhang, C., M.R. Fowler, N.W. Scott, G. Lawson and A. Slater. 2007. A TaqMan real-time PCR system for the identification and quantification of bovine DNA in meats, milks and cheeses. *Food Control*, **18**: 1149-1158.