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Application of a quadruplex PCR for the simultaneous detection of fraud and the presence of *Salmonella* sp. and *Listeria monocytogenes* in commercial samples of Marajó Cheese

Aplicação de uma PCR *quadriplex* para a detecção simultânea de fraude e da presença de *Salmonella* sp. e *Listeria monocytogenes* em amostras comerciais de Queijo do Marajó

Adrianne Maria Brito Pinheiro da Rosa[1]; Matheus Martins Ramos[2]; Ana Paula Presley Oliveira Sampaio[1]; Mylla Christy da Silva Dufossé[1]; Joelson Sousa Lima[3]; Talita Bandeira Roos[4]; Carina Martins de Moraes[4]*

Highlights

The technique is useful to investigate fraud and pathogenic contaminants in food.
Fraud involving the addition of cow's milk to Marajó Cheese was detected.
The presence of *Salmonella* sp. was identified in commercial samples of cheese.

Abstract

Bubaline cheese from the Marajó Archipelago has gained prominence for its nutritional value and unique flavor. However, due to the seasonal scarcity of buffalo milk, cases of fraud with cow's milk have been reported. Additionally, the cheese's rich nutrient content and the potential lack of hygienic-sanitary controls in its production make it a favorable substrate for pathogenic microorganisms, such as *Salmonella* sp. and *Listeria monocytogenes.* The objective of this study was to develop a multiplex PCR for the simultaneous detection and differentiation of fraud by bovine milk, through identification of buffalo and bovine DNA, as well as the detection of *Salmonella* sp. and *Listeria monocytogenes* in commercial samples of Marajó Cheese, aiming to support its application as a routine tool in sanitary

[1] PhD graduates of the Postgraduate Program in Animal Health in the Amazon , Universidade Federal do Pará, UFPA, Castanhal, PA, Brazil. E-mail: adriannemariabrito@gmail.com; anapaulasamppaio@gmail.com; mylladufosse@gmail.com
[2] Doctoral Student of the Graduate Program in Animal Health in the Amazon, UFPA, Castanhal, PA, Brazil. E-mail: mvmatheusmr@gmail.com
[3] Prof., PhD, Universidade da Amazônia, UNAMA, Castanhal, PA, Brazil. E-mail: joelsonbio@live.com
[4] Profas, PhD, Graduate Program in Animal Health in the Amazon, UFPA, Castanhal, PA, Brasil. E-mail: talitaroos@gmail.com; carinamoraes@ufpa.com.br
* Author for correspondence

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inspection. To conduct the experiment, 25 samples of buffalo cheese were collected at different time points. DNA was extracted from the samples and subjected to the proposed quadruplex PCR. Fraud was detected in 20% of the samples (5/25), and *Salmonella* in 8% (2/25), while *Listeria monocytogenes* was not detected. These findings confirm the presence of bovine milk in buffalo cheeses from the Marajó Archipelago and demonstrate that the developed quadruplex PCR may serve as an important tool for use by inspection agencies.
Key words: Food fraud. Hygienic-sanitary control. Microbiological contamination. Molecular methods. Pathogenic microorganisms.

Resumo

O queijo bubalino oriundo do Arquipélago do Marajó vem ganhando destaque graças as riquezas nutricionais e ao seu sabor único. Devido à baixa disponibilidade sazonal do leite bubalino, fraudes por adição de leite de vaca vêm sendo relatadas. Além disso, seus nutrientes e a possível falta de padrões higiênico-sanitário na produção tornam o referido queijo um substrato para diversos microrganismos patogênicos, como *Salmonella* sp. e *Listeria monocytogenes.* O objetivo do presente estudo foi desenvolver uma PCR multiplex para a detecção e diferenciação simultânea de fraude por adição de leite bovino, por meio da identificação de DNA de espécies bubalina e bovina, bem como da presença dos patógenos *Salmonella* sp. e *Listeria monocytogenes* em amostras comerciais de Queijo do Marajó, visando seu uso como ferramenta na rotina de fiscalização sanitária. Para a realização do experimento foram coletadas 25 amostras queijos de búfala, em períodos distintos. A extração de DNA das amostras foi realizada, seguida da aplicação da PCR quadriplex proposta. Foram detectadas as fraudes em 20% das amostras (5/25) e a presença de *Salmonella* em 8% das amostras (2/25), a presença de *Listeria monocytogenes* não foi detectada. Assim, concluímos que há fraude por adição de leite de vaca em queijos de búfala no Arquipélago Marajó, e que a PCR *quadriplex* apresentada pode ser uma ferramenta importante a ser utilizada pelos órgãos fiscalizadores.
Palavras-chave: Métodos moleculares. Fraude alimentar. Contaminação microbiológica. Microrganismos patogênicos. Controle higiênico-sanitária.

Introduction

In the Brazilian Amazon, buffalo milk production has both economic and social importance, and interest in its use for dairy products such as cheese has grown considerably (J.A.R. Silva et al., 2021). Within this context, the Marajó Archipelago stands out as a center for buffalo farming and artisanal dairy production, particularly for Marajó Cheese, which has gained regional and national appreciation, especially after receiving the Art Seal in 2019 and the Geographical Indication registration in 2021.

Marajó Cheese is traditionally made in the Archipelago of the same name, in the state of Pará, through family-based production using recipes passed down through generations, highlighting the region's historical and cultural heritage. It is produced from pure buffalo milk and/or a mixture with cow's milk, with a maximum allowed addition of 40%, provided that this is declared on the product label (Portaria No. 0418/2013 - ADEPARÁ, 2013).

Despite its cultural and economic importance, the limited seasonal availability of buffalo milk makes Marajó Cheese more susceptible to fraud, including adulteration, substitution of raw material, or inaccurate labeling. These issues have been reported by several authors (C.L. Silva et al., 2015; Cardoso et al., 2019).

Such practices are often employed to conceal quality deficiencies, artificially increase product weight, or mask undesirable changes, thereby maximizing profits (Decree No. 10.468, 2020; Visciano & Schirone, 2021). Beyond fraud, there is also a risk of contamination by pathogenic microorganisms such as *Listeria monocytogenes* and *Salmonella* sp., particularly because the cheese is traditionally made from raw milk (Figueiredo et al., 2011; Bittencourt et al., 2013).

One method used to detect both fraud and microbial contamination in food is the Polymerase Chain Reaction (PCR) and its variations. PCR is widely used due to its high sensitivity and specificity, as well as its rapid turnaround, which helps reduce time and cost in diagnostics (Li et al., 2023; Nesterova et al., 2024). Based on this, Lima et al. (2021) proposed a quadruplex PCR for the simultaneous detection of DNA from bovine, buffalo, *L. monocytogenes,* and *Salmonella* sp. in buffalo cheeses. Although this method showed experimental efficiency, it had not yet been tested on commercial samples, which may present specific challenges that affect its routine application in inspection procedures.

The aim of this study was to standardize a multiplex PCR for the simultaneous detection and differentiation of fraud through the addition of bovine milk, by identifying buffalo and bovine DNA, and detecting the presence of *Salmonella* sp. and *Listeria monocytogenes* in commercial samples of Marajó Cheese, with the goal of supporting its use as a routine tool in sanitary inspection.

Materials and Methods

The study involved the collection of 25 Marajó Cheese samples sold in municipalities in the Marajó Archipelago, state of Pará, Brazil, during the Amazon summer (October) in 2018 and 2019. The samples, representing 17 different brands available at the time, were obtained from commercial establishments in the municipalities of Cachoeira do Arari, Salvaterra, Soure, and Ponta de Pedras.

After acquisition, the samples were transported in their original packaging to the Food Hygiene and Quality Laboratory at the Federal University of Pará (UFPA) - Castanhal Campus, for the proposed molecular analyses. Each cheese sample was cataloged, and information such as brand, origin, type of cheese, and inspection seal was recorded.

To reproduce and standardize the method proposed by Lima et al. (2021), a model adulterated cheese was produced in triplicate, using a 1:1 ratio of bovine to buffalo milk. This experimentally adulterated cheese was then contaminated with approximately 3-5 colony-forming units (CFUs) of *Salmonella typhimurium* (ATCC 14028) and *Listeria monocytogenes* (ATCC 7644), and subsequently served as a positive control for PCR.

For experimental contamination, CFUs were added to 225 mL of brain-heart infusion (BHI) broth (HiMedia®, India), along with 25 g of the adulterated cheese. The mixture was

incubated at 37 ºC for 24 h. After incubation, 2 mL of the culture and approximately 2 g portions of the incubated cheese were collected for DNA extraction, following the protocol described by Darwish et al. (2009).

To verify the integrity of the extracted genetic material, the DNA samples were eluted in 30 µL of TE buffer solution (Tris-HCl-EDTA), subjected to 0.8% agarose gel electrophoresis (Inlab™), stained with a non-mutagenic dye (GelRed™, Biotium, California, USA), and visualized under ultraviolet light using a transilluminator (Gel Documentation System, Del Doc™, Bio-Rad®, California, USA), with image analysis performed using Total Lab TL Lab 5.2 software. The quality and concentration of the DNA were determined by spectrophotometry (NanoDrop® ND-1000 UV-Vis, Thermo Fisher Scientific Inc., Massachusetts, USA), with absorbance measured at 260/280 nm and concentrations expressed in ng/µL.

Subsequently, the 25 commercial cheese samples were cultured in BHI medium at 37 ºC for 24 h to recover any viable bacterial cells that might be present. From each culture, 2 mL aliquots and approximately 2 g of cheese were collected for DNA extraction, following the previously described protocol.

Multiplex PCR was carried out as proposed by Lima et al. (2021). The primers and their target regions are listed in Table 1.

Table 1
Primers used in PCR, target regions for amplification, and respective bibliographic references

Target	Primer (5′ → 3′)	Fragment size (pb)	Reference
Bovine (*Bos taurus*)	Forward: CTAGAGGAGCCTGTTCTATAATCGATA	346	López-Calleja et al. (2005)
	Reverse: AAATAGGGTTAGATGCACTGAATCCAT		
Buffalo (*Bubalus bubalis*)	Forward: CTAGAGGAGCCTGTTCTATAATCGATA	220	López-Calleja et al. (2005)
	Reverse: TTCATAATAACTTTCGTGTTGTTGGGTGT		
***Salmonella* spp.**	Forward (ST11): GCCAACCATTGCTAAATTGGCGCA	429	Aabo et al. (1993)
	Reverse (ST15): GGTAGAAATTCCCAGCGGGTACTGG		
Listeria monocytogenes	Forward (LM1): CTAAGACGCCAATCGAA	702	Border et al. (1990)
	Reverse (LM2): AAGCGCTTGCAACTGCTC		

The PCR reaction mixture included 0.4 pmol of each primer (LM1, LM2, SR11, ST15, Bov reverse, Buf reverse, and Bov-Buf forward), 100 mM Tris-HCl (pH 8.5), 500 mM KCl (2X Buffer), 2.4 mM $MgCl_2$, 10 mM dNTP mix, 2.5 U of Taq DNA Polymerase (Ludwig Biotec®, Brazil), approximately 80 ng of template DNA, and sterile Milli-Q water (q.s.) to a final volume of 25 µL. The reactions were performed in a thermal cycler (Applied Biosystems Veriti™ Thermal Cycler, California, USA), with the following steps: initial denaturation at 94 ºC for 5 min; 35 cycles of denaturation at 94 ºC for 30 s, primer annealing at 56 ºC for 45 s, and extension at 72 ºC for 45 s; followed by a final extension at 72 ºC for 5 min.

The resulting amplicons were subjected to electrophoresis on a 1.5% agarose gel (Inlab™, Brazil) in 0.5X TBE buffer, stained a non-mutagenic dye (GelRed™, Biotium, California, USA), and visualized under UV light. A 50-bp molecular weight marker (Ludwig Biotec®, Brazil) was used as a reference. The results were recorded using a transilluminator (Gel Documentation System, Del Doc™, Bio-Rad®, California, USA). Data were tabulated, and the absolute and relative frequencies of fraud and microbial contamination were calculated using Microsoft Excel™ 2024 (Microsoft Corporation, USA).

Results

Table 2 presents the information on sampling locations, cheese types (cream or butter), and the presence or absence of inspection seals on the analyzed products. Figure 1 illustrates the detection results for bovine DNA and the presence of pathogenic contaminants.

Regarding fraud detection, 20% of the samples (5/25), corresponding to five different brands, were found to be non-compliant: 4% (1/25) in the first sampling in October 2018 (brand E1, sample 5), and 16% (4/25) in the second sampling in October 2019 (brand F2, sample 12; brand N2, sample 19; brand M2, sample 14; and brand C2, sample 22), due to the presence of bovine DNA not indicated on the label. Most cases involved fraud by incorporation, since buffalo DNA was also detected. However, in one brand (M2, sample 14), only bovine DNA was identified, indicating fraud by substitution of the raw material.

As for microbial contamination, 8% (2/25) of the samples one from each collection period tested positive for *Salmonella* sp. DNA (brand K1 – Salvaterra – no seal; and brand A2 – Soure – cream-type and no seal), indicating contamination. Both contaminated samples were unsealed and not fraudulent. No *L. monocytogenes* DNA was detected in any of the samples analyzed.

Table 2
Data on collections carried out in two different periods, with information on location, cheese type, inspection seal, and results of DNA detection by PCR

Sample	Brand	Location	Type	Inspection	Authenticity	*Salmonella* sp.	*Listeria monocytogenes*
1st collection							
1	A1	Soure	-	No seal	Bubaline DNA	Absent	Absent
2	B1	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
3	C1	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
4	D1	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
5	**E1**	**Soure**	**Cream**	**No seal**	**Bubaline and Bovine DNA**	**Absent**	**Absent**
6	F1	Salvaterra	Cream	SIE	Bubaline DNA	Absent	Absent
7	G1	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
8	H1	Cachoeira do Arari	-	No seal	Bubaline DNA	Absent	Absent
9	I1	Cachoeira do Arari	-	SIE	Bubaline DNA	Absent	Absent
10	J1	Cachoeira do Arari	Butter	SIE	Bubaline DNA	Absent	Absent
11	**K1**	**Salvaterra**	**-**	**No seal**	Bubaline DNA	**Present**	**Absent**
2nd collection							
12	**F2**	**Salvaterra**	**Cream**	**SIE**	**Bubaline and Bovine DNA**	**Absent**	**Absent**
13	L2	Cachoeira do Arari	-	No seal	Bubaline DNA	Absent	Absent
14	**M2**	**Soure**	**Butter**	**SIE**	**Bovine DNA**	**Absent**	**Absent**
15	H2	Cachoeira do Arari	-	No seal	Bubaline DNA	Absent	Absent
16	I2	Cachoeira do Arari	Butter	SIE	Bubaline DNA	Absent	Absent
17	D2	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
18	G2	Soure	Creme	SIE	Bubaline DNA	Absent	Absent
19	**N2**	**Cachoeira do Arari**	**-**	**No seal**	**Bubaline and Bovine DNA**	**Absent**	**Absent**
20	B2	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
21	**A2**	**Soure**	**Cream**	**No seal**	Bubaline DNA	**Present**	**Absent**
22	**C2**	**Soure**	**Cream**	**SIE**	**Bubaline and Bovine DNA**	**Absent**	**Absent**
23	O2	Soure	Cream	SIE	Bubaline DNA	Absent	Absent
24	P2	Salvaterra	Cream	SIE	Bubaline DNA	Absent	Absent
25	Q2	Pontas de Pedras	Cream	SIE	Bubaline DNA	Absent	Absent

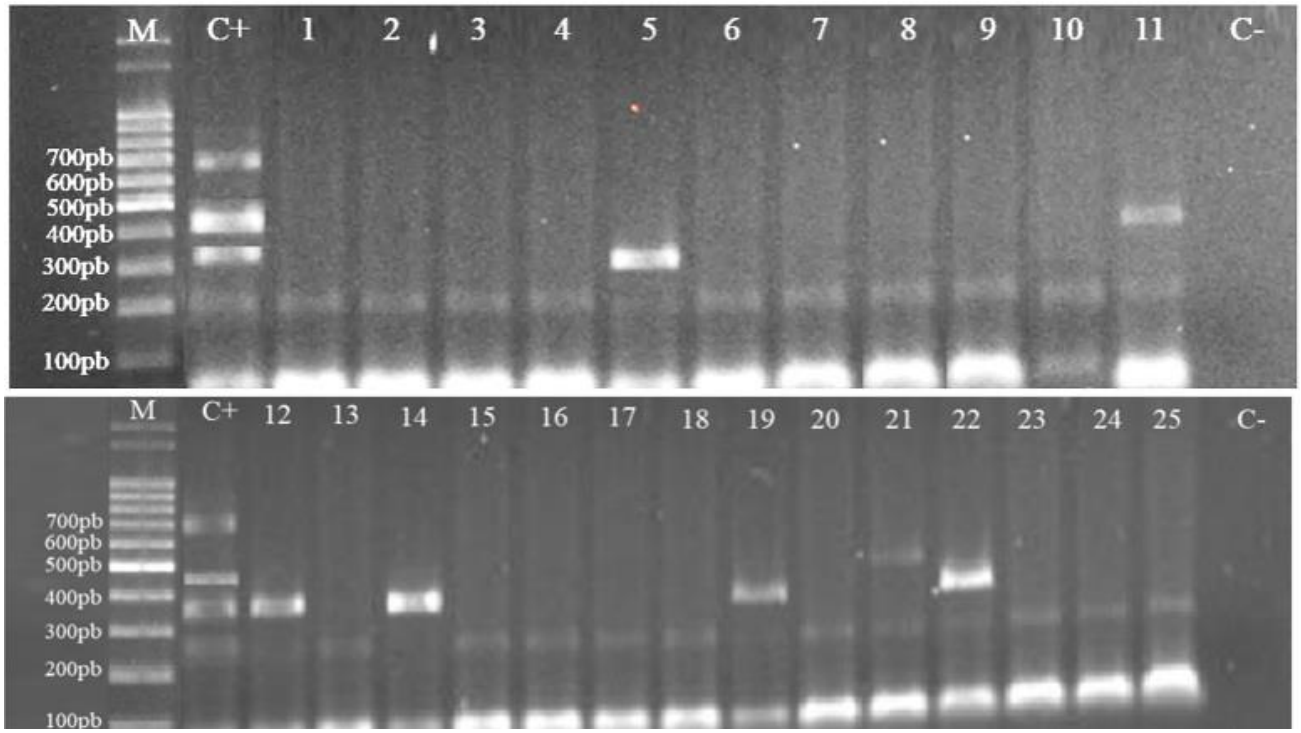

Figure 1. 1.5% agarose gel electrophoresis showcasing the products of a PCR from the first sample collection for the simultaneous detection of *Bos taurus* and *Bubalus bubalis, Listeria monocytogenes,* and *Salmonella* sp. from cheese samples marketed in the Marajó Archipelago, where: M – 100-bp molecular weight marker; C+ - positive control with amplification of fragments of approximately 346 bp (*Bos taurus*) and 220 bp (*Bubalus bubalis*), 702 bp (*Listeria monocytogenes*) and 429 bp (*Salmonella* sp.); C- - negative control; 1 to 4, 6 to 10, 13, 15 to 18, 20, and 23 to 25 – detection of DNA of the *Bubalus bubalis* species in the analyzed samples; 5, 12, 19 and 22 - simultaneous detection of DNA from the *Bubalus bubalis* and *Bos taurus* species in the analyzed samples; 11 and 21 - simultaneous detection of DNA from the *Bubalus bubalis* and *Salmonella* sp. species in the analyzed samples; 14 - detection of DNA from the *Bos taurus* species in the analyzed samples.

Discussion

Marajó Cheese, in addition to its nutritional and economic relevance, represents a key cultural symbol of the Amazon region. With potential for expansion into new markets, it may drive sustainable buffalo milk production. However, such expansion depends on guaranteeing product authenticity and sanitary quality (Kamimura et al., 2019).

The state regulation governing Marajó Cheese was established in 2013 (Portaria No. 0418/2013 - ADEPARÁ, 2013), while RIISPOA the Brazilian regulatory framework for animal-based food production was updated in 2020 [Ministério da Agricultura, Pecuária e Abastecimento, (2017)], livestock and Supply, Decree No. 10.468, 2020). Under the current law, cheeses produced from milk of different animal species must state the percentage of the predominant milk source on the label,

which allows for the legal sale of mixed-milk products.

The results of this study confirm that fraud in Marajó Cheese is a reality. The detection of bovine DNA in samples without proper labeling represents a violation of both state and federal regulations (Decree No. 10.468, 2020; Portaria No. 0418/2013 - ADEPARÁ, 2013). These data confirm the findings of Abedini et al. (2023), who noted that cheese fraud is widespread due to the high market value of these products and has been observed globally in various cheese types in recent years. Therefore, it is suggested that Marajó Cheese producers adapt to this situation by formalizing it as a product containing both buffalo and bovine milk.

As shown in Table 2, fraudulent samples coincided with periods of low buffalo milk productivity (October 2018 and 2019), which likely led producers to supplement with cow's milk without declaring it on labels. Cardoso et al. (2019) also reported increased fraud rates in buffalo cheeses in the Marajó archipelago during the dry season, when buffalo milk availability decreases. In their study, of the nine samples analyzed, 100% of those collected during the rainy season and 33% during the dry season were found to be fraudulent.

Furthermore, buffalo cheese fraud is common in these regions, even though buffalo raw materials are abundant. C.L. Silva (2015), analyzing 44 samples of buffalo cheese sold in municipalities in the states of Pará and Amapá, Brazil, identified fraud in 13.6% of the commercial samples. These findings indicate the urgent need for public initiatives aimed at raising awareness and regulating production in the region to ensure compliance with current health regulations.

PCR and its variations are commonly used individually to detect either fraud or microbial contamination in food. However, Lima et al. (2021) developed and standardized a quadruplex PCR protocol capable of simultaneously detecting DNA from bovine and buffalo species, as well as *Salmonella* spp. and *L. monocytogenes.* This technique allows for the detection of adulteration by bovine milk at concentrations as low as 1%. Moreover, the authors demonstrated that bacterial DNA could be amplified after just 6 h of culture when contamination involved 1 CFU, and immediately (0 h) with 2 or 3 CFUs.

The same authors (Lima et al., 2021) also emphasized the potential for bacterial contamination in fraudulent cheese production practices, as manipulation and the addition of foreign components to raw material can compromise product quality. In the present study, both cow's milk adulteration and the presence of *Salmonella* sp. were detected in commercial samples, reinforcing the effectiveness and reliability of the proposed technique.

Another important factor influencing Marajó Cheese production is the sanitary status of buffalo herds raised extensively in the Archipelago and the precarious hygienic conditions of artisanal cheese manufacturing, which traditionally uses raw milk (Bittencourt et al., 2013; Figueiredo et al., 2011). These elements, combined with the high nutritional value of buffalo milk, raise concerns about potential contamination by pathogenic microorganisms (Agostini et al., 2012). This reinforces the importance of investigating the presence of such agents in Marajó Cheese, given the public health risks involved. In this study, *Salmonella* sp. and *L. monocytogenes,* whose absence is required by current Brazilian

legislation (Instrução Normativa No. 60, 2019, 2019; Portaria No. 0418/2013 - ADEPARÁ, 2013), were chosen as target pathogens.

Guzman-Hernandez et al. (2016) used PCR to detect microorganisms in commercial cheeses and reported contamination by *Salmonella* spp., attributed to poor hygiene at the collection sites. In the present study, inadequate manufacturing or storage conditions may also have contributed to contamination, especially in cheeses without inspection seals, underlining the importance of official inspection to prevent the commercialization of foods that pose a risk to public health.

Although the presence of *L. monocytogenes* in cheeses has been documented in various regions (Allaion et al., 2021; Gérard et al., 2018; Maia et al., 2019), there are no previous records in the Marajó Archipelago. According to Mudadu et al. (2022), factors such as competing microbiota, water activity, and pH may limit the survival of this pathogen. In the present study, the presence of lactic acid bacteria may have contributed to its inhibition, emphasizing the need to consider environmental and biological factors in food safety assessments.

Azevedo et al. (2021) warn of the risk of adulteration in buffalo dairy products due to the high production cost of buffalo milk. Additionally, Montgomery et al. (2020) point out that issues related to cheese, including contamination and fraud, are more frequent than in other dairy products. Therefore, it is essential to implement efficient control methods considering costs and available resources, and to improve tools capable of simultaneous analysis to enhance risk prevention and safeguard consumer health.

A limitation of this study is the use of conventional PCR, which only yields qualitative results and does not differentiate between viable and non-viable microorganisms. Moreover, factors such as DNA degradation during processing, cross-contamination, and the presence of PCR inhibitors (e.g., fats, proteins, polysaccharides) can affect the accuracy of the results. Nevertheless, the method remains relevant and provides essential information on the presence of both pathogens and adulterants in the analyzed samples.

The present research highlights the potential of this tool for detecting both fraud and pathogens in cheese. While *L. monocytogenes* was not found in the commercial samples, no previous studies have reported simultaneous detection of fraud and these pathogens in field samples from the Marajó Archipelago. The methodology implemented here presents an excellent methodological alternative for analyzing buffalo cheeses and can be extrapolated to other dairy products available on the market. Finally, given the recurrent detection of bovine milk in cheeses labeled as buffalo in Marajó, it is recommended that the responsible authorities consider officially recognizing Marajó Cheese as a mixed-milk product.

Conclusion

This study confirms the occurrence of fraud by incorporation or substitution of bovine milk in buffalo cheeses from the Marajó Archipelago, as well as contamination by *Salmonella* spp. The previously standardized quadruplex PCR method was successfully applied to field samples and is recommended

for use by regulatory agencies and official laboratories as a complementary tool in routine inspections. Furthermore, the findings highlight the need for public policies aimed at strengthening health surveillance and promoting the regularization and inspection of animal-origin products in the region to ensure food safety and protect consumer health.

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References

Aabo, S., Rasmussen, O. F., Roseen, L., Sørensen, P. D., & Olsen, J. E. (1993). Salmonella identification by the polymerase chain reaction. *Molecular and Cellular Probes, 7*(3), 171-178. doi: 10.1006/mcpr.1993.1026

Abedini, A., Salimi, M., Mazaheri, Y., Sadighara, P., Sani, M. A., Assadpour, E., & Jafari, S. M. (2023). Assessment of cheese frauds, and relevant detection methods: a systematic review. *Food Chemistry: X, 19,* 100825. doi: 10.1016/j.fochx.2023.100825

Agostini, C., Kreling, C. S., Bustamante, I. C., Fº., Souza, C. F. V. de, Biolchi, V., & Pozzobon, A. (2012). Detecção de *Listeria monocytogenes* pela técnica da reação em cadeia da polimerase (PCR) em amostras de leite bovino *in natura. Revista do Instituto de Laticínios Cândido Tostes, 67*(389), 15-20. doi: 10.5935/2238-6416.20120073

Allaion, J. R., Barrionuevo, K. G., & Franco, B. D. G. de M. (2021). Assessing the microbiological safety parameters of minas artisanal cheese samples in retail environments in São Paulo, Brazil. *Applied Sciences, 11*(19), 9331. doi: 10.3390/app11199331

Azevedo, B. T., Vercesi, A. E., Fº., Gutmanis, G., Verissimo, C. J., Katiki, L. M., Okino, C. H., Sena Oliveira, M. C. de, & Giglioti, R. (2021). New sensitive methods for fraud detection in buffalo dairy products. *International Dairy Journal, 117,* 105013. doi: 10.1016/j.idairyj.2021.105013

Bittencourt, R. H. F. P. de M., Cortez, M. A. S., Márcico, E. T., Rosa, R. M. S. S., Taxi, C. M. A. D., Faturi, C., & Ermita, P. A. N. (2013). Caracterização de Requeijão Marajoara e Minas Frescal produzidos com leite de búfalas no Estado do Pará, Brasil. *Ciência Rural, 43*(9), 1687-1692. doi: 10.1590/S0103-84782013005000110

Border, P. M., Howard, J. J., Plastow, G. S., & Siggens, K. W. (1990). Detection of Listeria species and *Listeria monocytogenes* using polymerase chain reaction. *Letters in Applied Microbiology, 11*(3), 158-162. doi: 10.1111/j.1472-765X.1990.tb00149.x

Cardoso, G. V. F., Oliveira, A. C. do S. de, Silva, A. S. da, Araújo, W. S. C., Dantas, V. V., Silva, J. B. da, Lima, J. S., Nunes, E. do S. C. de L., Guimarães, R. J. de P. S., & Roos, T. B. (2019). Detection of fraud by addition cow's milk in cheese buffalo and its connection with seasonality. *Revista Brasileira de Ciência Veterinária, 26*(4), 152-157. doi: 10.4322/rbcv.2019.027

Darwish, S. F.; Allam, H. A.; Amin, A. S. (2009). Evaluation of PCR assay for detection of cow's milk in water buffalo's milk. *World Applied Sciences Journal, 7*(4), 461–467. https://www.researchgate.net/publication/237796736_Evaluation_of_PCR_Assay_for_Detection_of_Cow's_Milk_in_Water_Buffalo's_Milk

Decree No. 10.468, of August 18, 2020. Amends Decree Nº. 9.013, of March 29, 2017, which regulates Law No. 1.283, of December 18, 1950, and Law Nº. 7.889, of November 23, 1989, on the regulation of industrial and sanitary inspection of products of animal origin. *Diário Oficial da União,* Brasília, 2020. https://www.planalto.gov.br/ccivil_03/_ato2019-2022/2020/decreto/d10468.htm

Figueiredo, E. L., Lourenço Júnior, J. de B. L., Toro, M. U., & Lima, S. C. G. de. (2011). Marajó cream-type cheese: physicochemical and sensory parameters. *Revista do Instituto de Laticínios Cândido Tostes, 66*(378), 26-33. https://www.revistadoilct.com.br/rilct/article/view/152

Gérard, A., El-Hajjaji, S., Niyonzima, E., Daube, G., & Sindic, M. (2018). Prevalence and survival of Listeria monocytogenes in various types of cheese - a review. *International Journal of Dairy Technology, 71*(4), 825-843. doi: 10.1111/1471-0307.12552

Guzman-Hernandez, R., Contreras-Rodriguez, A., Hernandez-Velez, R., Perez-Martinez, I., Lopez-Merino, A., Zaidi, M. B., & Estrada-Garcia, T. (2016). Mexican unpasteurised fresh cheeses are contaminated with *Salmonella* spp., non-O157 Shiga toxin producing *Escherichia coli* and potential uropathogenic *E. coli* strains: a public health risk. *International Journal of Food Microbiology, 237,* 10-16. doi: 10.1016/j.ijfoodmicro.2016.08.018 105013

Instrução Normativa No. 60, of December 23, 2019. Establishes microbiological standards for food. *Ministério da Agricultura, Pecuária e Abastecimento. Diário Oficial da União,* Brasília, DF, 2019. https://cvs.saude.sp.gov.br/zip/U_IN-MS-ANVISA-60_231219.pdf

Kamimura, B. A., Magnani, M., Luciano, W. A., Campagnollo, F. B., Pimentel, T. C., Alvarenga, V. O., Pelegrino, B. O., Cruz, A. G., & Sant'Ana, A. S. (2019). Brazilian artisanal cheeses: an overview of their characteristics, main types and regulatory aspects. *Comprehensive Reviews in Food Science and Food Safety, 18*(5), 1636-1657. doi: 10.1111/1541-4337.12486

Li, B., Yu, M., Xu, W., Chen, L., & Han, J. (2023). Comparison of PCR techniques in adulteration identification of dairy products. *Agriculture, 13*(7), 1450. doi: 10.3390/agriculture13071450

Lima, J. S., Sampaio, A., Dufossé, M. C. S., Rosa, A., Sousa, P. F. M., Silva, J. B., Cardoso, G. V. F., Moraes, C. M., & Roos, T. B. (2021). Standardization of a rapid quadruplex PCR method for the simultaneous detection of bovine, buffalo, *Salmonella* spp., and *Listeria monocytogenes* DNA in milk. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 73,* 781-790. doi: 10.1590/1678-4162-12218 105013

López-Calleja, I., Alonso, I. G., Fajardo, V., Rodríguez, M. A., Hernández, P. E., García, T., & Martín, R. (2005). PCR detection of cows' milk in water buffalo milk and mozzarella cheese. *International Dairy Journal, 15*(11), 1122-1129. doi: 10.1016/j.idairyj.2004.12.003

Maia, D. S. V., Haubert, L., Würfel, S. de F. R., Kroning, I. S., Cardoso, M. R. de I., Lopes, G. V., Fiorentini, Â. M., & Silva, W. P. da. (2019). *Listeria monocytogenes* in sliced cheese and ham from retail markets in southern Brazil. *FEMS Microbiology Letters, 366*(22), fnz249. doi: 10.1093/femsle/fnz249

Ministério da Agricultura, Pecuária e Abastecimento (2017). *Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal – RIISPOA* (Approved by Decree No. 9.013). *Diário Oficial da República Federativa do Brasil.* https://www.gov.br/agricultura/pt-br/assuntos/mpa/legislacao/legislacao-geral-da-pesca/decreto-no-9-013-de-29-03-2017.pdf/view

Montgomery, H., Haughey, S. A., & Elliott, C. T. (2020). Recent food safety and fraud issues within the dairy supply chain (2015-2019). *Global Food Security, 26,* 100447. doi: 10.1016/j.gfs.2020.100447105013

Mudadu, A. G., Piras, G., Melillo, R., Salza, S., Cau, S., Virgilio, S., Meloni, D., & Mele, P. (2022). Survival of naturally contaminating *Listeria monocytogenes* in commercial mediterranean-style dry fermented sausages during Storage. *Journal of Food Protection, 85*(11), 1576-1583. doi: 10.4315/JFP-22-023

Nesterova, E., Morozova, P., Gladkikh, M., Kazemzadeh, S., & Syromyatnikov, M. (2024). Molecular methods for detecting microorganisms in beverages. *Beverages, 10*(2), 46. doi: 10.3390/beverages10020046

Portaria No. 0418/2013 – ADEPARÁ. (2013). Technical regulation for the production of Queijo do Marajó and other provisions. *Agência de Defesa Agropecuária do Estado do Pará (ADEPARÁ).* https://www.legisweb.com.br/legislacao/ ?id=252036

Silva, C. L., Sales, G. A., Santos Neto, J. G., Silva, J. da, Lima, S. C. G. de, Leite, F. P. L., Lima, E. do S. C. de, Moraes, C. C. G. de, Roos, T. B., & Moraes, C. M. de. (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. https://docs.bvsalud.org/biblioref/ses-sp/2015/ses-32090/ses-32090-5948.pdf

Silva, J. A. R., Garcia, A. R., Almeida, A. M. de, Bezerra, A. S., & Brito Lourenço, J. de., Jr. (2021). Water buffalo production in the Brazilian Amazon Basin: a review. *Tropical Animal Health and Production, 53*(3), 343. doi: 10.1007/s11250-021-02744-w

Visciano, P., & Schirone, M. (2021). Food frauds: global incidents and misleading situations. *Trends in Food Science & Technology, 114,* 424-442. doi: 10.1016/j.tifs.2021.06.010