

Article - Food/Feed Science and Technology

# Assessment of Microbial Contamination in Products of Animal Origin: Stretched-curd Cheese, Yogurt and Fresh Sausage

**Denise Maria de Souza Mendes<sup>1</sup>**  
<https://orcid.org/0000-0002-8105-0282>

**Bruno Vinícius Bastos<sup>2</sup>**  
<https://orcid.org/0000-0003-2066-2426>

**Cassia Ribeiro Koki<sup>2</sup>**  
<https://orcid.org/0000-0002-9673-7108>

**Daniele Bach<sup>2</sup>**  
<https://orcid.org/0000-0001-9208-4624>

**Gisele Kirchbaner Contini<sup>2</sup>**  
<https://orcid.org/0000-0003-1369-3515>

**Lorena Alves de Mattos<sup>2</sup>**  
<https://orcid.org/0000-0002-8905-0949>

**Mateus Flório Cubo<sup>2</sup>**  
<https://orcid.org/0000-0003-1916-0306>

**Priscila Arcoverde Wosiack<sup>2</sup>**  
<https://orcid.org/0000-0001-6594-8538>

**Rafaela Gomes da Silva<sup>2</sup>**  
<https://orcid.org/0000-0002-5434-2909>

**Raíssa Arantes Barboza<sup>2</sup>**  
<https://orcid.org/0000-0003-2714-0508>

**Taynara Pacheco Valério<sup>2</sup>**  
<https://orcid.org/0000-0003-4566-6773>

**Luiz Gustavo Lacerda<sup>1,2</sup>**  
<https://orcid.org/0000-0002-4689-5431>

**Luís Antônio Esmerino<sup>3</sup>**  
<https://orcid.org/0000-0003-2495-3490>

**Alessandro Nogueira<sup>1,2\*</sup>**  
<https://orcid.org/0000-0001-7671-3056>

<sup>1</sup>State University of Ponta Grossa, Department of Food Engineering, Ponta Grossa, Paraná, Brazil; <sup>2</sup>State University of Ponta Grossa, Graduate Program in Food Science and Technology, Ponta Grossa, Paraná, Brazil; <sup>3</sup>State University of Ponta Grossa, Department of Clinical and Toxicological Analysis, Ponta Grossa, Paraná, Brazil.

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\*Correspondence: [alessandronog@yahoo.com.br](mailto:alessandronog@yahoo.com.br); Tel.: +55-42-32203775

## HIGHLIGHTS

- Coliforms at 35 °C and 45 °C can suggest the lack of sanitary practices;
- Water in the processing can be a source of contamination and should be monitored;
- *S. aureus* can be an indicator of the quality in good manufacturing practices during food processing.

**Abstract:** Animal products are sources of microbiological contamination when the process has hygienic-sanitary control fails. Therefore, this work aims the evaluation of the pathogenic microorganisms presented in samples from the Brazil southern region of yogurt (N = 101), stretched curd cheese (N = 31), fresh sausage (N = 22) and processing water (N = 63). Analyses of coliforms at 45 °C, *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli* were performed. Analysis indicated processing water is an important contamination source to be monitored, because the majority of samples presented results above the regulation limits. Thermal treatment and fermentation such as stretched curd cheese and yogurt appeared to be more stable

against contamination during processing. In this study, for coliforms at 45 °C, only one cheese sample and 12% of total yogurt samples exceeded the Brazilian legislation limit. None of sausage samples presented any contamination. On the other hand, values found in both processing water and dairy products indicated failures in application and monitoring of good manufactured practices.

**Keywords:** products of animal origin, Coliforms, *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli*.

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## INTRODUCTION

In 2017, Brazilian production of animal protein was one of the largest worldwide. Among them, milk and pork with 22.7 and 3.3 million tons, respectively, were the main sources [1, 2]. Products from raw protein are widely and produced and consumed, such as cheese with 755 thousand tons in 2018 [2], yogurt with 840 thousand tons and sausages, including fresh sausages, with 1.5 million tons in 2016 [3].

Recent data shows that a rate of 1 in 10 people gets illness from contaminated food daily while this can be the cause of 420,000 deaths per day [4,5]. Between 2009 and 2018, water was the main source that caused food outbreaks in Brazil (21.1%). Milk and dairy products were in fourth place with 7.8% and meat sausages in 14<sup>th</sup> place with 2% [6]. According to Fung and coauthors [5], bacteria are the most serious and lethal agents that cause foodborne illness. One of the main microorganisms groups used as contamination indicators are the coliforms [7]. Among the 80 outbreaks identified in the laboratory in 2018, *Escherichia coli* stands out for being the etiological agent in 27.5% of cases [6].

Microbiological contaminants can be found from processing water, raw materials and failures during processing, distribution or storage [8,9]. Coliforms at 45 °C are fecal indicators, and *E. coli* is a microorganism indicator of this source of contamination. Total coliforms, *S. aureus* and *Salmonella* sp. are normally related to poor hygienic and sanitary conditions, inadequate handling and spoiled raw materials [10,11].

Problems found in dairy products, like cheese and yogurt, may originate from high microbial counting. It can be due raw milk, failures and contaminants after heat treatment, contamination in the started culture or curd and poor hygiene in the utensils, equipment and environment used in the production [12,10,13,14]. Cheese spoilage also can happens in the brine and curd stages [15]. Contamination in meat sausage products is related to bad raw material quality and good manufacturing failures [16,17].

Pathogenic microorganisms can lead to inflammation in the gastrointestinal tract, complications regarded to several toxic substances production, generalized infections and in some cases, it can leads to death [18]. *Salmonella* sp. is responsible for generalized infections and the major cause of hospitalizations and deaths worldwide [19, 20]. The symptoms by this contamination are diarrhea, fever, headache and abdominal pain [21,22]. Other bacteria found in contaminated food are pathogenic *E. coli*, which leads to diarrhea, urinary tract infections and even meningitis [23]. According to Tranter [24], *Staphylococcus aureus* causes food poisoning because it produces extracellular toxins, leading to symptoms such as nausea, vomiting, abdominal pain, and diarrhea within 2 to 6 hours after ingestion of contaminated food.

Therefore, due to the importance of pathogenic food control and the constant claims about food safety all over the world, this work aims to evaluate the microbiological food quality in animal products (stretched-curd cheese, yogurt, and fresh sausage), from Brazil Southern region.

## MATERIAL AND METHODS

### Material

Samples of yogurt (n = 101), stretched-curd cheese (n = 31), fresh sausage (n = 22) and water applied in the processing (n = 63) were supplied by companies located in the southern region of Brazil. Defective samples on the package or expired date have been discarded. All reagents used in this study were of analytical grade.

### Methods

In the yogurt samples, coliform analysis was performed at 45 °C. Analysis of Coliforms, *Staphylococcus* sp. and *Salmonella* sp. were performed in the samples of stretched-curd cheese and fresh sausage. The analysis of coliforms, aerobic mesophylls and *E. coli* were performed in the water samples.

## Preparation of samples

For the food analyses, 25 g or mL of the samples were crushed and homogenized in 225 mL of 0.1% sterile peptone water (HiMedia Laboratories, Mumbai, India) previously sterilized in an autoclave at 121°C for 15 min. From this dilution  $10^{-1}$  the serial dilutions were performed up to  $10^{-4}$ . For water analysis, there was no need for prior dilution.

## Salmonella sp. analysis

*Salmonella* sp. is a qualitative analysis, performed according to ABNT NBR ISO 6579/2007 [25], and the result is given as positive or negative in 25 g of the sample. The sample was diluted to  $10^{-1}$  in buffered peptone distilled water (ADPT) 1% (HiMedia Laboratories, Mumbai, India), incubated at room temperature between 4 and 6 hours and then at 35 °C for 24 hours. 1 mL of ADPT culture was transferred aseptically in tubes with tetrathionate, 0.15 mL of brilliant green solution and 0.2 mL of iodinated iodine solution and in tubes with selenite medium. The tubes were incubated at 41 °C for 24 and 48 hours. After incubation, they were plated in *Salmonella-Shigella* (SS) medium (HiMedia Laboratories, Mumbai, India) and brilliant green agar (BG) at 35 °C for 24 hours. After the incubation period, the presence of colonies and their characteristics were verified. Colonies in the SS medium that were yellow, transparent, small, with or without black spots were plated in the modified Rugai medium and identified biochemically [26]. The same procedure was performed for colonies that presented small size, pink and transparent coloration at BG medium. Colonies were also submitted to agglutination reaction using *Salmonella* polyvalent antisera (Probac, São Paulo, Brazil) [26].

## Staphylococcus aureus analysis

*S. aureus* were determined to cheese and sausage products [26]. The plate counting method was performed from sample preparation, inoculum incubation and counting from presumptive colonies. *S. aureus* strains were identified by confirmatory tests. From the typical colonies confirmation tests presented by Silva [27], three were applied: (a) catalase test, (b) coagulase test and (c) termonuclease test. Among these, test (b) was modified, replacing rabbit EDTA plasma with rabbit saline plasma (0.1%).

## Coliforms analysis

Coliforms count at 35 °C and 45 °C were performed by the most probable number method (MPN). 10 ml of the sample were inoculated in inverted Durham tubes with concentrated Laurel Sulfate Tryptose (LST) broth (UroGen, New York, USA). Tubes were incubated at 35 °C for 48 hours. From each positive tube, in the presence of gas, an aliquot was taken and inoculated in inverted Durham tubes with brilliant green 2% bile broth (VB) (Kasvi, Sao Jose dos Pinhais, Brazil). These tubes were incubated at the same conditions described. The same procedure were applied for *Escherichia coli* (EC) broth (Kasvi, Sao Jose dos Pinhais, Brazil) where tubes were incubated at 45 °C for 24 hours in a water bath. The number of positives tubes were analyzed according to MPN chart and expressed as described by APHA [28]. From each tube with EC broth, a sample was plated in Teague or BEM. The plates were incubated at 35 °C for 24 hours. After the incubation, the typical colonies were identified and then inoculated in modified Rugai tubes at 35 °C for 24 hours and identified biochemically [26]. The results were analyzed on a proper table.

## Aerobic mesophilic analysis

Aerobic mesophilic bacteria analysis was performed using the leakage plate method. In sterile Petri dishes, 0.4 mL triphenyltetrazolium chloride (TTC) (Vetec Quimica, Rio de Janeiro, Brazil) and 1 ml diluted sample were transferred. The plate counting agar (APC) at 45 °C (Kasvi, São José dos Pinhais, Brazil) was added to the plate and carefully homogenized by slow rotational movements. The plates were incubated at  $35 \pm 1$  °C for 48 hours. After this period, the colonies were counted (multiplied by dilution) and the results obtained were expressed in CFU/g or mL. Colonies between 30 and 300 were considered significant.

## RESULTS

Water samples were not conformed to Brazilian regulations regarding the absence of coliforms in 100 mL [29,30]. Presence of thermal tolerant bacteria were found in all the samples at 35 °C and in 97% of samples at 45 °C (Table 1).

**Table 1.** Microbiological assessment of water samples (N=63) applied into food processing.

Coliforms at 35 °C (NMP/g)*	Coliforms at 45 °C (NMP/g)**	Aerobic Plate Count (UFC/mL)***
< 1.1 (49)	Absent (2)	50 (1)
6.9 (3)	< 1.1 (53)	< 10 <sup>2</sup> (8)
9.2 (3)	6.9 (3)	< 2.2 x 10 <sup>2</sup> (2)
12 (2)	9.2 (1)	5.4 x 10 <sup>2</sup> (1)
16 (1)	12.1 (2)	1.0 x 10 <sup>3</sup> (1)
23 (5)	16 (1)	2.9 x 10 <sup>3</sup> (1)
-	> 23 (1)	6.3 x 10 <sup>3</sup> (2)
-	-	9.3 x 10 <sup>3</sup> (1)
-	-	2.3 x 10 <sup>4</sup> (1)
		nd (45)

Note: Values in parentheses refer to number of samples analyzed. \*Limit value for Coliforms at 35 °C: absence/100 mL; \*\* limit value for coliforms at 45 °C: absence/100 mL; \*\*\* limit value for Aerobic Plate Count: 5x10<sup>2</sup> UFC/mL. nd: not determined.

Table 2 shows only one sample of cheese within the legislation limits for coliforms at 45°C (5 x 10<sup>3</sup> NMP/g).

**Table 2.** Microbiological assessment of stretched curd cheese (N= 26), fresh sausage (N=22) and yoghurt (N=101).

Microbiological limits	Stretched curd cheese	Fresh sausage	Yoghurt
	< 3 (18)	< 3 (11)	< 3 (72)
	4 (2)	4 (3)	4 (10)
	11 (1)	9 (3)	9 (6)
Coliforms at 45 °C (NMP/g)*	43 (3)	15 (1)	23 (4)
	240 (1)	23 (2)	43 (3)
	> 1100 (1)	460 (1)	75 (2)
	-	1100 (1)	93 (1)
	-	-	150 (1)
	-	-	240 (1)
<i>Staphylococcus aureus</i> (UFC/g)**	< 10 <sup>2</sup> (24)	< 10 <sup>2</sup> (22)	-
	nd (2)	-	-
<i>Salmonella</i> sp***	Absent (24)	Absent (21)	-
	nd (2)	nd (1)	-

Note: Values in parentheses refer to number of samples analyzed. \*Limit value for Coliforms at 45 °C: 10<sup>3</sup> NMP/g; \*\* limit value for *Staphylococcus aureus*: < 10<sup>3</sup> UFC/g; \*\*\* limit value for *Salmonella*: absence in 25 g. nd: not determined.

Sausage samples (Table 2) were within the Brazilian legislation established limits for *Staphylococcus aureus* (5 x 10<sup>2</sup> UFC/g).

## DISCUSSION

According to Alves and coauthors [31], water contaminations can occur due to failures in the public collection system, also because of the bad hygiene and maintenance of the water reservoir. In countryside area there is a lack of treatment and protection of artesian wells, streams, rivers and dams [32]. As well as to the Brazilian regulations, the samples were not conformed in the United States legislation, which states that total coliforms should not surpass the limit of 5 % from total samples analyzed in a month [33]. Aerobic Plate Count presented 9.5 % of results above the established limit (5x10<sup>2</sup> UFC/mL), being 10<sup>4</sup> UFC/mL the highest value found. Ćirić and coauthors [34] claim that recontamination can occur after water treatment and chlorination. Aerobic Mesophilic Bacteria can recover from chemical effects and lack of nutrients, being able to proliferate in treated water distributed.

All the samples of stretched curd cheese, fresh sausage and yoghurt were according the Brazilian legislation limits for *Staphylococcus aureus* (max. 10<sup>3</sup> UFC/g) and *Salmonella* spp. (absence) [35]. The European Union limits for coliforms count in cheese is ten times lower than Brazilian limits (100 UFC/g) [36]. Prates and coauthors [37] and Marinheiro and coauthors [38] also found absence of *Salmonella* spp in stretched curd cheeses. It can be related to thermal process since the curd (5.2 pH) is stretched under water at high temperature (55-88 °C) and can reach 40 to 70 °C during 5 to 10 minutes [39].

Sausage samples analyzed had results similar to studies from Daguer and coauthors [40] where *Staphylococcus aureus* contamination was not found (max 5 x 10<sup>2</sup> UFC/g) among meat samples obtained from Paraná state. These results are due adequate control and sanitary processes [35] during prepare of meat products. Only one sample was found near the maximum limit allowed (5 x 10<sup>3</sup> NMP/g) for coliforms at

45 °C (Table 2). The EU establishes a 500 UFC/g limit for *E. coli*. Coagulase-positive staphylococcal (max.  $5 \times 10^3$  UFC/g) and *Salmonella* sp. (absent in 25 g) were not detected among contamination [33, 41]. However, *Salmonella* sp was found in other studies in the percentage of 28.3% [42], 24.4% [43] and 17.1% [44] of samples analyzed. Fresh pork products usually do not go under thermal or fermentative processing hence the microbiological quality of raw material it is a critical control point [45,46].

Within the yogurt samples, 12% presented higher values than the established limits in the legislation for coliforms at 45 °C. The current Brazilian legislation regulates a maximum of 10 MPN/g [47], as well the EUA and EU are 10 UFC/100 g to coliforms and 10 UFC/mL to enterobacteria, respectively [48,36]. Fermentation process that occurs in yogurt promotes the medium acidification, which can inhibit contamination by microorganisms as coliforms [13]. According to Emiliano and coauthors [49], presence of coliforms is an unsatisfactory quality indicator of hygiene practices during processing and packing. National and international regulations establish good manufacturing practices for food services and home cooking, in order to ensure consumers health safety as well food quality [50,51]. These practices are essential in order to avoid food contamination. Therefore, microbiological analysis are fundamental for food safety assurance and for each food category there are specific and mandatory analyses [52].

## CONCLUSION

Coliforms at 35 °C and 45 °C results suggests that are a lack of sanitary practices at some point of the processes analyzed. It can occur because failures in important process stages, inadequate food manipulation by employees and bad water quality. Contamination by mesophilic aerobic bacteria found in processing water samples confirmed this. Improvements in good manufacturing practices are necessary as well supervision and controlling of these processes in order to ensure the efficiency and food safety. To coliforms at 45°C, all fresh sausage samples reached the limits established by the legislation. Only one cheese sample and 12% of yogurt samples were above the regulations limits allowed. All fresh sausage and cheese samples were within the parameters established for *S. aureus* and *Salmonella* sp. Through *S. aureus* results it can be noticed the quality in good manufacturing practices during food processing (stretched-curd cheese and fresh sausage) manufactured by industries from South Brazil in the samples evaluated in this work.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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