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Microbiological quality of curd cheese purchased at a street market in the municipality of Maceió-AL

JESUS, Évelly Vitória Oliveira de[1]; SILVA, Livia Ribeiro da[2]; SILVA, Maria Clariana da[3]; SILVA, Paula Cibelly Vilela da[4]; MONTALDO, Yamina Coentro[5]; SANTOS, Tania Marta Carvalho dos[6]

[1] 0009-0009-4115-0420, Federal University of Alagoas (*Universidade Federal de Alagoas*), researcher, Brazil, vitoriaevelly61@gmail.com;

[2] 0009-0000-6850-7383, Federal University of Alagoas (*Universidade Federal de Alagoas*), student and researcher, Brazil, ribeirolivia1508@gmail.com;

[3] 0009-0001-7405-7830, Federal University of Alagoas (*Universidade Federal de Alagoas*), student and researcher, Brazil, maria.clariana@ceca.ufal.br;

[4] 0000-0003-2157-4698, Federal University of Alagoas (*Universidade Federal de Alagoas*), doctoral and researcher, Brazil, paulacibelly17@gmail.com;

[5] 0000-0003-1444-8642, Federal University of Alagoas (*Universidade Federal de Alagoas*), teacher and researcher, Brazil, ycmzte11@gmail.com;

[6] 0000-0002-1816-7840, Federal University of Alagoas (*Universidade Federal de Alagoas*), teacher and researcher, Brazil, taniamarta2@gmail.com.

ABSTRACT

The objective of this study was to determine aspects related to the microbiological quality of curd cheese samples sold at a street market in Maceió-AL. The presence of total and thermotolerant coliforms, total mesophiles, *Salmonella spp*. and *Escherichia coli* has been evaluated. The samples were submitted to analysis to determine the MPN/g of total (35°C/24 h) and thermotolerant (45°C/24 h) coliforms. For *Salmonella* detection, pre-enrichment with 1% peptone water, enrichment in selenito-cystine and Tetrathionate broth, and plating on Hektoen agar and Salmonella-Shigella agar to determine presence/absence of *Salmonella*. For the detection of total mesophiles, serial dilution and plating on Count Standard Agar (PCA) and incubation at 28°C were used. All samples showed growth at different levels, the presence of coliforms at 35°C was < 3 NMP/g in sample 1, 3 NMP/g in sample 2 and 21 NMP/g in sample 3. The samples showed growth of coliforms 45 °C, 21 MPN/g in sample 1, 75 MPN/g in sample 2 and 43 MPN/g in sample 3. The presence of *Salmonella spp*. was not detected in the samples. The average number of mesophiles was 4.33 x 109, 4.34 x 109 and 4.36 x 109 CFU/g. The evaluated samples are within the standards recommended by regulatory agencies.

RESUMO

Objetivou-se determinar aspectos relativos à qualidade microbiológica de amostras de queijo coalho comercializados em uma feira livre em Maceió-AL. Avaliou-se a presença de coliformes totais e termotolerantes, mesófilos totais, *Salmonella spp*. e *Escherichia coli*. As amostras foram submetidas a análise para determinação do NMP/g de coliformes totais (35°C/24 h) e termotolerantes (45°C/24 h). Para detecção de *Salmonella*, pré-enriquecimento com água peptonada a 1%, enriquecimento em selenito-cistina e caldo Tetrationato, e plaqueamento em ágar Hektoen e ágar Salmonella-Shigella para determinar presença/ausência de *Salmonella*. Para detecção dos mesófilos totais, foi utilizado a diluição em série e plaqueamento em Ágar Padrão Contagem (PCA), e incubação em 28°C. Todas as amostras apresentaram crescimento em diferentes níveis, a presença de coliformes 35°C foi de < 3 NMP/g na amostra 1, 3 NMP/g na amostra 2 e 21 NMP/g na amostra 3. As amostras apresentaram crescimento de coliformes 45°C, 21 NMP/g na amostra 1, 75 NMP/g na amostra 2 e na amostra 3 de 43 NMP/g. Não foi detectada a presença de *Salmonela spp*. nas amostras. A média de mesófilos foi de 4,33 x 109, 4,34 x 109 e 4,36 x 109 UFC/g. As amostras avaliadas estão nos padrões recomendados pelos órgãos de regulação.

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Introduction

According to Martinelli (2022), for Northeastern culture curd cheese is a centuries-old fruit that to this day represents a symbol of tradition, persistence and bravery. Over the years, the production of this cheese has been linked to the survival of the participants in the production and commercial system, being responsible for keeping the producer, the seller and the buyer afloat. It has proved to be the sole or main source of income for many families in the agreste and sertão, as it is mostly produced in these regions.

Food obtained through artisanal processes is extremely likely to be contaminated, due to the use of raw materials from unsafe sources, poorly sanitized or contaminated utensils, preparation under improper storage conditions and marketing at inadequate temperatures, all of which contribute to increasing the risk of contamination (Oliveira *et al.,* 2010).

When people consume milk and dairy products that have not undergone any kind of treatment, such as pasteurization, they are vulnerable to a wide range of microorganisms and toxins capable of causing food poisoning. This fact must be taken into account, as the majority of the population who consume milk are children and the elderly (Miller, 2008). The presence of a high number of micro-organisms in food represents a risk for many immunosuppressed individuals who, due to low immunity, are more likely to die (Silva, 2017).

Food not produced in accordance with Good Manufacturing Practices, regulated by the National Health Surveillance Agency (ANVISA), is contaminated by various types of microorganisms. Bacteria are considered to be the biggest agents of food contamination. This contamination can cause diseases generically known as food-borne illnesses (FBD) (Alves, 2012).

DTAs can be prevented if food preparation follows Good Manufacturing Practices (GMP), avoiding cross-contamination and allowing correct sanitization according to each type of food. Furthermore, after 12 hours of preparation, the food must be stored in a suitable place and at a temperature that does not allow the growth of micro-organisms. It is of the utmost importance to train employees who handle food in accordance with GMP, thus preventing contamination (Silva, 2017).

The aim of this study was to evaluate the microbiological quality of curd cheese in order to verify and quantify the presence of 35°C and thermotolerant coliforms, Salmonella spp., Escherichia coli and total mesophiles. The Introduction should contextualize the proposal of the manuscript, justification and objectives.

Material and Methods

Place of research

The samples were obtained from three different local sellers at a street market in the Tabuleiro dos Martins neighborhood, in the municipality of Maceió, state of Alagoas. They were packed in plastic bags, identified and transported in a thermal box to the Microbiology Laboratory at the Campus of Engineering and Agricultural Sciences (CECA), located at 9°27'58.7" south latitude and 35°49'35.1" west latitude, of the Federal University of Alagoas (*Universidade Federal de Alagoas* - UFAL).

Figure 1.

Sample analysis site in Rio Largo - AL (Coordinates: 9°27'58.7" S and 35°49'35.1" O).

Note: Google® Earth Pro.

Sample preparation

Twenty-five grams of curd cheese from each sample were weighed and macerated separately in previously autoclaved porcelain crucibles (Figure 2). The samples were suspended in 225 ml of 1% peptone water, previously sterilized at 121 °C in an autoclave, resulting in a dilution of 10^{-1} from which subsequent dilutions were made up to 10^{-5}. The entire preparation procedure was carried out under aseptic conditions.

Figure 2.

Cheese samples weighed and separated (A), samples macerated in crucibles (B) and samples suspended in peptone water (C).

Note: Author's archive.

Sample analysis

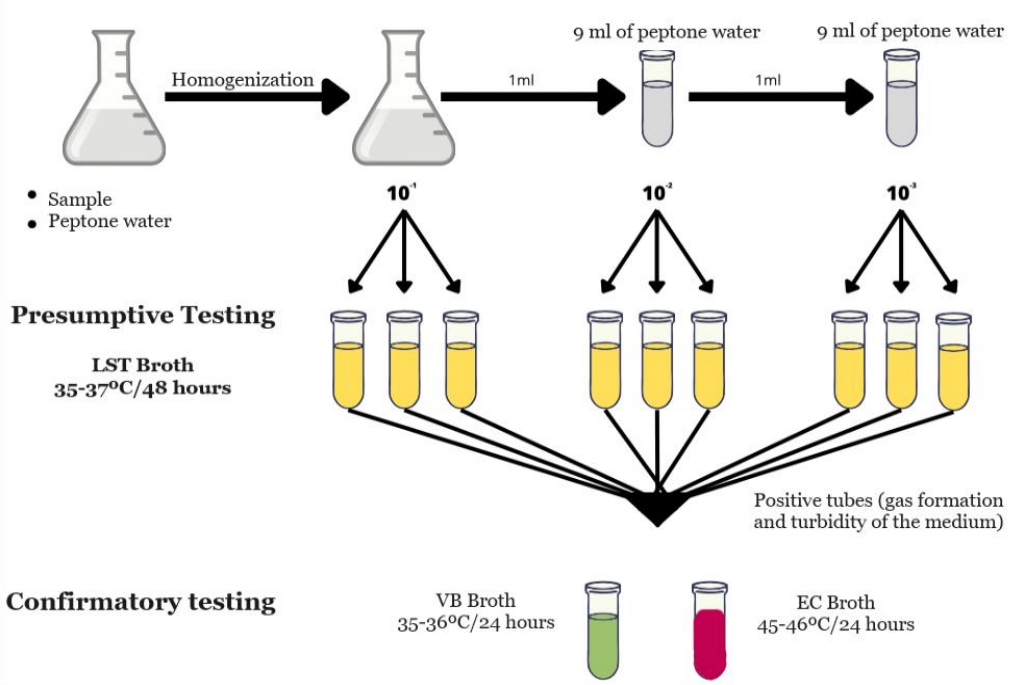
Determination of MPN/g of total and thermotolerant coliforms

The multiple tube technique was used to determine the MPN of total coliforms, according to the methodology described by the Brazilian Association of Technical Standards (*Associação Brasileira de Normas Técnicas* - ABNT, 1991). This technique comprises two distinct phases: The presumptive test, in which the cells recover and detect the presence of lactose-fermenting microorganisms, and the confirmatory test, in which the actual population of total and thermotolerant coliforms is determined.

The presumptive test used 3 series (dilutions 10^{-1} to 10^{-3}) of 3 tubes containing LST (Lauryl Sulphate Tryptose) broth and Durham tubes (Figure 3). The cultures were incubated in an oven at 37°C for 24-48 hours. After incubation, a reading was taken and the test was considered positive if there was turbidity and obvious gas production identified in the Durham tube.

Figure 3.

Flowchart of the analyses to determine the amount of coliforms.



Note: Prepared by the author (2023), adapted from Dias (2015).

Confirmatory test for total coliforms 35 °C

Each positive sample was sown in test tubes containing lactose-bile-green brilliant 2% and Durham tubes, and the cultures were incubated at 35-36°C for 24 hours. Tubes that showed turbidity and gas production were considered positive. The most probable number (MPN/g) table was used to quantify the results.

Confirmatory test for thermotolerant coliforms 45°C

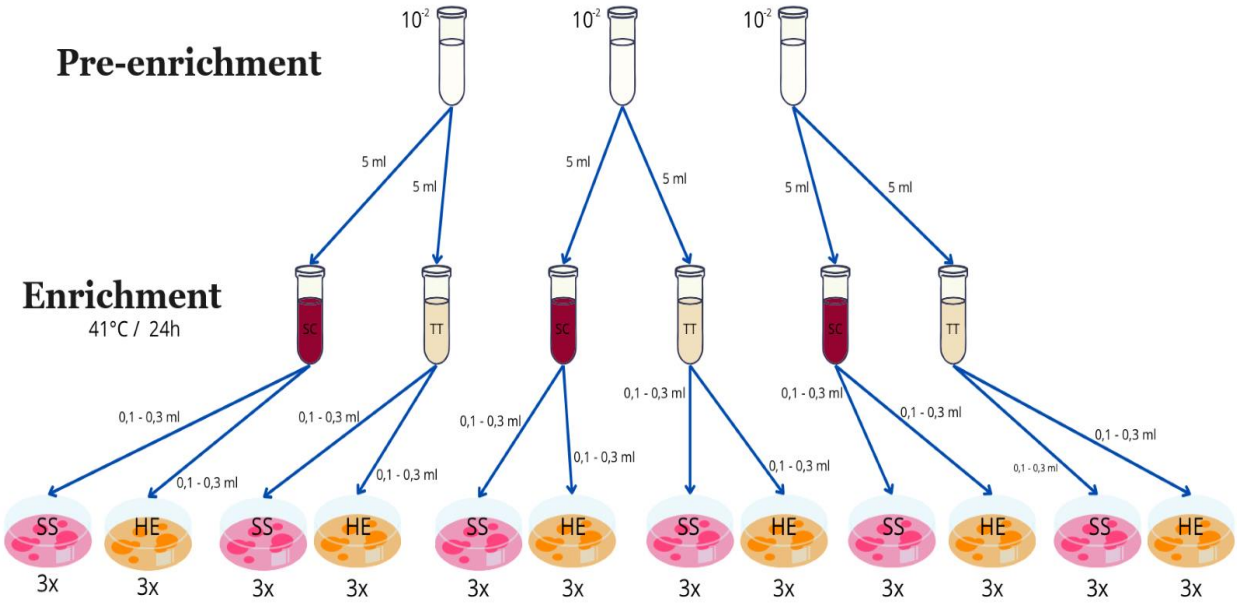
Each positive sample was sown in test tubes containing EC (*Escherichia coli*) broth and the cultures were incubated at 45-46°C for 24 hours. Tubes with gas production in the Durham tubes and turbidity of the medium were considered positive. The most probable number (MPN) table was used to quantify the results.

Test for *Salmonella spp.*

To detect *Salmonella*, the samples were pre-enriched with 1% peptone water, then enriched in selenite-cystine and Tetrationate broth and, for isolation, Hektoen agar and Salmonella-Shigella agar (Figure 4).

Figure 4.

Flowchart of the analysis steps for detecting the presence of *Salmonella spp*.
in the cheese samples purchased.



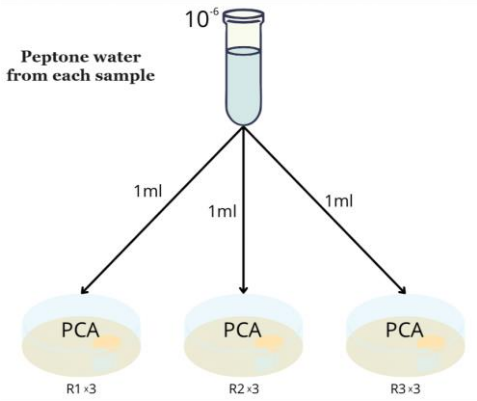
Note: Prepared by the author (2023).

Mesophilic microorganisms

From the 10^{-6} dilution, one ml was plated in depth in Petri dishes containing 15 ml of PCA culture medium, with three repetitions of each sample. After plating, the plates were incubated inverted for 48 hours at 32°C. The plates with counts between 30 and 300 CFU were selected for colony counting (Figure 5).

Figure 5.

Flowchart for counting mesophilic colonies in PCA.



Note: Prepared by the author (2023).

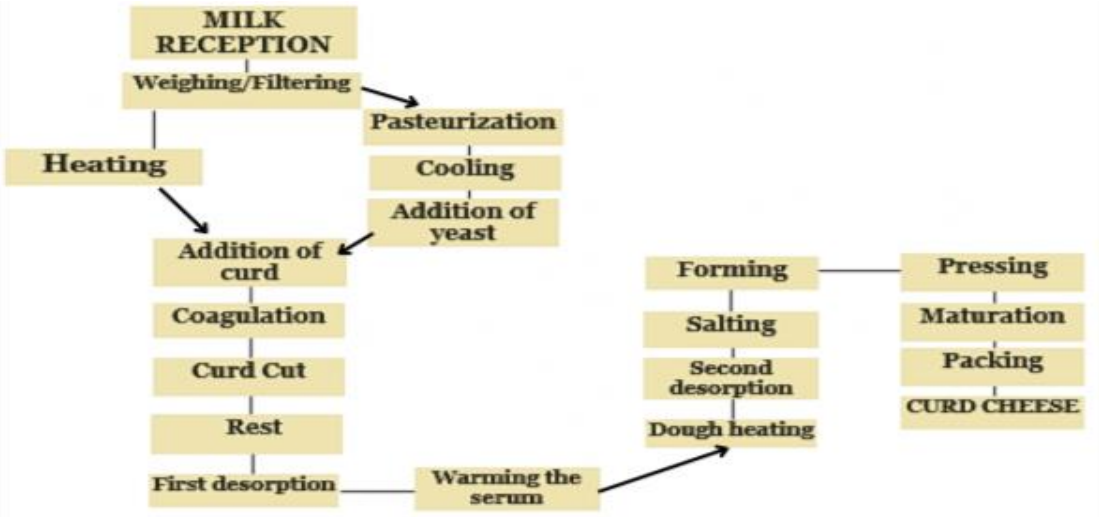
Results and Discussion

If the curd cheese has quantities of 35 °C coliforms, thermotolerant coliforms, Salmonella spp. and other microorganisms outside the limit stipulated by the legislation, it indicates that there was contamination during processing, processing, transportation or storage. In small farms with artisanal processes, the cheese is heated and then curd is added. As it does not undergo pasteurization or similar heat treatment, there is a great risk that pathogenic microorganisms will continue to be present during processing.

In industrialized production, the milk undergoes thermal processes to guarantee the safety of the product, in this case curd cheese. Storage at inadequate temperatures and handling during marketing can lead to contamination (Figure 6).

Figure 6.

Flowchart of curd cheese production.



Note: Prepared by the author (2023).

Thus, checking the microbiological quality of food produced from animal or plant products is necessary to prevent outbreaks of STDs caused by poor hygiene during preparation, processing and storage.

All the samples analyzed showed growth at different levels (Table 1). This study showed results below the limits established by RDC No. 12 of January 2nd, 2001, with a maximum of 5x10² NMP/g.

Table 1.

Determination of the most probable number (MPN/g) of total coliforms and thermotolerant coliforms.

Cheese samples	Total coliforms 35 °C (NMP/g)	Thermotolerant coliforms 45°C (NMP/g)
Sample 1	< 3 NMP/g	21 NMP/g
Sample 2	3 NMP/g	75 NMP/g
Sample 3	21 NMP/g	43 NMP/g

Note: Survey data (2023).

The results of the present study were below the limits established by the legislation, with a maximum of 5x10² MPN/g. Even though they are within the limits, the results declare that the raw material and/or consequently its derivatives have levels of fecal contamination due to the hygienic-sanitary conditions during the preparations for milk milking, during milking in inappropriate places, the storage of milk without cooling or the production of derivatives without the pasteurization process or similar heat treatments.

The presence below the levels has also been reported in other studies, for example in the research carried out by Nunes (2017), contamination by coliforms at 35°C was found in 100% of the 30 samples analyzed, 24 of these samples equivalent to 80% of the sampling showed a count above 103 NMP/g. In 60% of the samples analyzed for thermotolerant coliforms, contamination below the limit established by the legislation was considered acceptable. Taking into account the thermotolerant coliform results, the presence of Escherichia coli was confirmed in 14 (46.67%) of the 30 samples analyzed.

Similarly, in a study carried out by Santos (2019), the samples from all the batches analyzed showed growth for 35°C and thermotolerant coliforms. With regard to the quantification of total coliforms in the samples, the result was 150 NMP/g in batch 1, 350 NMP/g in batch 2 and 350 NMP/g in batch 3. The thermotolerant coliform count in this study was 121 NMP/g in batch 1, 93 NMP/g in batch 2 and 150 NMP/g in batch 3.

Furthermore, in a study carried out by Oliveira et al (2010), of the 42 samples analyzed, 40 (95.24%) did not comply with the microbiological standards established by RDC 12, and were classified as unsuitable for human consumption. With regard to thermotolerant coliforms, it

was found that 80.95% (34/42) of the samples had values of more than 500 NMP/g, above the permitted limit, with 76.47% (26/34) of the curd cheeses having state inspection, 100% (1/1) having federal inspection and 100% (7/7) not having inspection.

Salmonella spp. was found to be absent from the three curd cheese samples, as there was no growth of Salmonella spp. on the plates with the selective media, *Salmonella-Shigella* (SS) agar and Hektoen (HE) agar. With regard to the quantification of total mesophiles found at the end of the analysis, all the samples showed growth (Table 2).

Table 2.

Quantification of mesophilic Colony Forming Units per gram (UFC/g) of curd cheese samples.

Cheese samples	Mesophiles (UFC/g)
Sample 1	4,33 x 10^9 UFC/g
Sample 2	4,34 x 10^9 UFC/g
Sample 3	4,36 x 10^9 UFC/g

Note: Survey data (2023).

In research carried out by Nunes (2017), there was growth in all the samples of artisanal curd cheese analyzed, with an average of 2.9x 10^5 UFC/g and counts that reached 1.9x 10^6 UFC/g. Analyzing the microbiological quality of curd cheese, Evangelista-Barreto *et al*. (2016) found an average count of 5.9 x 10^8 CFU/g, which was lower than in the present study. The authors linked the presence of these micro-organisms in curd cheese to the use of unsafe milk, poor hygiene of utensils, processing in unsanitary conditions, storage and marketing in unsuitable locations and temperatures.

Final considerations

The lack of technical knowledge on the part of small producers to prevent the existence of coliforms and other micro-organisms is often the villain of dairy farms, due to contamination during processing, processing, transportation or storage. Although the results of this study show levels of contamination, the cheese samples evaluated are within the standards recommended by the regulatory bodies. This is evidence of an improvement in the exchange of hygiene and health information and good practices in the production of milk and dairy products between producers and professionals.

REFERENCES

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT)-MB 3463. *Bactérias coliformes totais, coliformes fecais e Escherichia coli em alimentos: determinação pelo número mais provável (NMP)*. Rio de Janeiro, 1991. p. 7.

ALVES, A. R. F. *Doenças alimentares de origem bacteriana*. Universidade Fernando Pessoa -Faculdade de Ciências da Saúde. Dissertação de Mestrado em Ciências Farmacêuticas, Porto, 2012.

BRASIL. ANVISA. *RDC nº 12 de 02 de janeiro de 2001*. Diário Oficial da União, de 10 de janeiro de 2001A. Disponível em: <https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2001/res0012_02_01_2001.html>

DIAS, J.D.N. et al. A*valiação das Condições Higiênico-Sanitárias de Leite Cru e Queijo Coalho Comercializados em Mercados Públicos no Norte do Piauí*. Saúde e Pesquisa, Piauí, v. 8, n. 2, p. 277-284, 28 set. 2015.

EVANGELISTA-BARRETO, N. S. SANTOS, G. C. F. SOUZA, J. F. S. BERNARDES, F. S. SILVA, I. P. *Queijos artesanais como veículo de contaminação de Escherichia coli e estafilococos coagulase positiva resistentes a antimicrobianos*. Revista Brasileira de Higiene e Sanidade Animal (v.10, n.1) p. 55 – 67, jan - mar (2016).

MARTINELLI, L. *O Queijo Coalho do Nordeste do Brasil: Desafios e possibilidades na construção de uma cadeia de valor cultural na visão de especialistas*. 2022. 164 f. Dissertação (Mestrado em Memória Social e Patrimônio Cultural) – Instituto de Ciências Humanas, Universidade Federal de Pelotas, Pelotas, 2022

MILLER, N. B. *Perfil do consumo de leite de derivados lácteos no município de Colatina ES. 2008*. p 72. Monografia de conclusão de curso (DEFESA E VIGILÂNCIA SANITÁRIA ANIMAL). Programa de pós-graduação QUALITTAS. Universidade Castelo Branco. 2008.

NUNES, G. D. S. *Qualidade microbiológica e perfil de resistência aos antimicrobianos do queijo de coalho artesanalmente produzido*. 2017. 73 p. Dissertação (Pós-Graduação Zootecnia, na área de Produção Animal (Nutrição e produção de alimentos) — Universidade Federal do Piauí.

OLIVEIRA, K. A.; NETO, J. E.; PAIVA, J.E.; MELO, L.E.H. *Qualidade microbiológica do queijo de coalho comercializado no município do Cabo de Santo Agostinho, Pernambuco, Brasil*. Arq. Inst. Biol., São Paulo, v.77, n.3, p.435-440, jul./set., 2010.

SANTOS, J. G. *Análise microbiológica do queijo coalho produzido por usuários da fazenda da esperança em Lagarto – Sergipe*. 2019. Monografia de conclusão de curso (Graduação em Farmácia) - Universidade Federal de Sergipe, Lagarto, 2019.

SILVA, V. B. DA. *Análise microbiológica de alimentos envolvidos em surtos de doenças transmitidas por alimentos ocorridos na macrorregião de Sorocaba de 2011 a 2015*. Programa de Aprimoramento Profissional, Sorocaba, 2017. Disponível em: <https://pesquisa.bvsalud.org/portal/resource/pt/ses-35838>.