

# Microbiological quality of cookies produced by family agro-industries of Solidarity Enterprises

## Qualidade microbiológica de biscoitos produzidos por agroindústrias familiares de Empreendimentos Solidários

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### ABSTRACT

**Introduction:** The “sequilho” is a popular biscuit in Brazil, usually produced by hand by family agro-industries. However, many times, due to the lack of Good Manufacturing Practices (GMP), these foods present a risk of contamination by pathogens, becoming potential carriers of Foodborne Diseases (FBD). **Objective:** To evaluate the microbiological quality of biscuits produced by family agro-industries of Solidarity Economic Enterprises in Bahia. **Method:** Between August and October 2022, 14 batches of biscuits were evaluated, consisting of 5 samples, totaling 70 samples, from 5 family agro-industries located in the municipalities of Santo Antônio de Jesus, São Felipe, Mutuípe, Teolândia, and Valença, in the State of Bahia, Brazil. Microbiological analysis was performed using the rapid Petrifilm™ plate count method (3M Company) for *Salmonella* sp. (AOAC 2014.01), *Escherichia coli* (AOAC 991.14), Molds and yeasts (AOAC 997.02), Coagulase-positive Staphylococci (AOAC 2003.07), and the traditional method for quantification of *Bacillus cereus* (AOAC 980.31). **Results:** 13 batches (92.9%) showed satisfactory quality. Of these, 9 (69.2%) presented satisfactory results with acceptable quality and 4 (30.8%) were satisfactory with intermediate quality. In addition, one batch (7.1%) was considered unsatisfactory because it had a *B. cereus* count above the limit allowed by health legislation. **Conclusions:** The biscuits evaluated had a low contamination rate, not representing a risk of the transmission of FBD, thus being suitable for human consumption. However, it is suggested the elaboration of public policies that involve the health education of the handlers, increase of production and valorization of the regional culture.

**KEYWORDS:** Food Microbiology; Identity and Quality Standard for Products and Services; Good Manufacturing Practices

### RESUMO

**Introdução:** O sequilho é um biscoito popular no Brasil, geralmente produzido artesanalmente por agroindústrias familiares. Entretanto, muitas vezes, devido à falta de boas práticas de fabricação (BPF), os alimentos assim produzidos apresentam risco de contaminação por agentes patogênicos, tornando-se potenciais veiculadores de Doenças de Transmissão Hídrica e Alimentar (DTHA). **Objetivo:** Avaliar a qualidade microbiológica de biscoitos produzidos por agroindústrias familiares de Empreendimentos Econômicos Solidários da Bahia. **Método:** Entre agosto e outubro de 2022, avaliouse 14 lotes de biscoito, compostos por 5 amostras, totalizando 70 amostras, advindos de 5 agroindústrias familiares situadas nos municípios de Santo Antônio de Jesus, São Felipe, Mutuípe, Teolândia e Valença, no estado da Bahia, Brasil. Realizou-se uma análise microbiológica por meio do método rápido de contagem em placas Petrifilm™ (3M Company) de *Salmonella* sp. (AOAC 2014.01), *Escherichia coli* (AOAC 991.14), bolores e leveduras (AOAC 997.02) e Estafilococos coagulase positiva (AOAC 2003.07) e pelo método tradicional para quantificação de *Bacillus cereus* (AOAC 980.31). **Resultados:** 13 lotes (92,9%) revelaram qualidade satisfatória. Desses, nove (69,2%) apresentaram resultado satisfatório com qualidade aceitável e quatro (30,8%) resultaram satisfatórios



com qualidade intermediária. Ademais, um lote (7,1%) foi considerado insatisfatório por apresentar contagem de *B. cereus* acima do limite permitido pela legislação sanitária. **Conclusões:** Os biscoitos avaliados apresentaram baixo índice de contaminação, não representando risco para a veiculação de DTSA, sendo, assim, adequados ao consumo humano. Entretanto, sugere-se a elaboração de políticas públicas que envolvam a educação sanitária dos manipuladores, incremento da produção e valorização da cultura regional.

**PALAVRAS-CHAVE:** Microbiologia de Alimentos; Padrão de Identidade e Qualidade de Biscoitos; Boas Práticas de Fabricação

## INTRODUCTION

Family agro-business is a social and economic enterprise, usually made up of a farmer and their family who, together, join forces with the aim of providing a source of income or supplementing it. For this reason, they work by transforming products from family farming into more elaborate by-products, with the aim of diversifying and adding value to the products produced<sup>1</sup>.

According to Nichele and Waquil<sup>2</sup>, the aspects that qualify a rural family agro-industry are its location in rural areas, the use of simple machinery, raw materials of its own origin or from neighbors, the use of artisanal processes in the production chain, and, to a large extent, the use of family labor. Furthermore, when several families, usually close to each other, come together, a solidarity economic enterprise (EES) can emerge.

The term EES, according to Gaiger<sup>3</sup>, was included in Brazil in the mid-1990s, a period that coincided with economic initiatives recognized for their associative nature and cooperation and self-management practices. Still according to the author<sup>3</sup>, this type of economy presents itself as an alternative for generating work and income for the most diverse social categories, including family agro-industries, and adopts the principle of solidarity, self-sufficiency, and seeks to guarantee the social permanence of its members.

It was for this reason that farmers from communities located in the municipalities of Santo Antônio de Jesus, São Felipe, Mutuípe, Teolândia, and Valença, in the state of Bahia, Brazil, saw the sale of cookies, popularly known in the region as “sequilhos”, as an opportunity for economic growth.

Thus, although it has a major social impact, due to various factors, such as the lack of advanced technology, inadequate transportation and storage, incipient infrastructure, and insufficient handling practices, the final products from this commercial sector are often compromised in terms of sensory and microbiological quality<sup>4,5,6</sup>.

According to Martin and De Dea Lindner<sup>7</sup>, the microbiological quality of food is related to the presence of microorganisms that can cause undesirable changes in the sensory characteristics of food, as well as pathogenic organisms that can directly or indirectly affect human health through water and foodborne diseases (FBD). These diseases, according to Brandão<sup>8</sup>, are important causes of morbidity and mortality, emerging as a potential economic and public health obstacle worldwide.

With this in mind, when looking to increase production with a view to marketing, there is a set of rules called good manufacturing practices (GMP) that must be adopted by these family agro-industries in order to ensure that the products they offer meet the quality and safety standards required by current health legislation<sup>9</sup>. In Brazil, the National Health Surveillance Agency (ANVISA) is responsible for creating the legislation that should guide the GMP of each establishment that produces and/or sells food, with the aim of maintaining the quality standard of the products, in order to guarantee the health of the population, reducing expenses with hospital admissions and providing greater safety and contentment to the final consumer<sup>10</sup>.

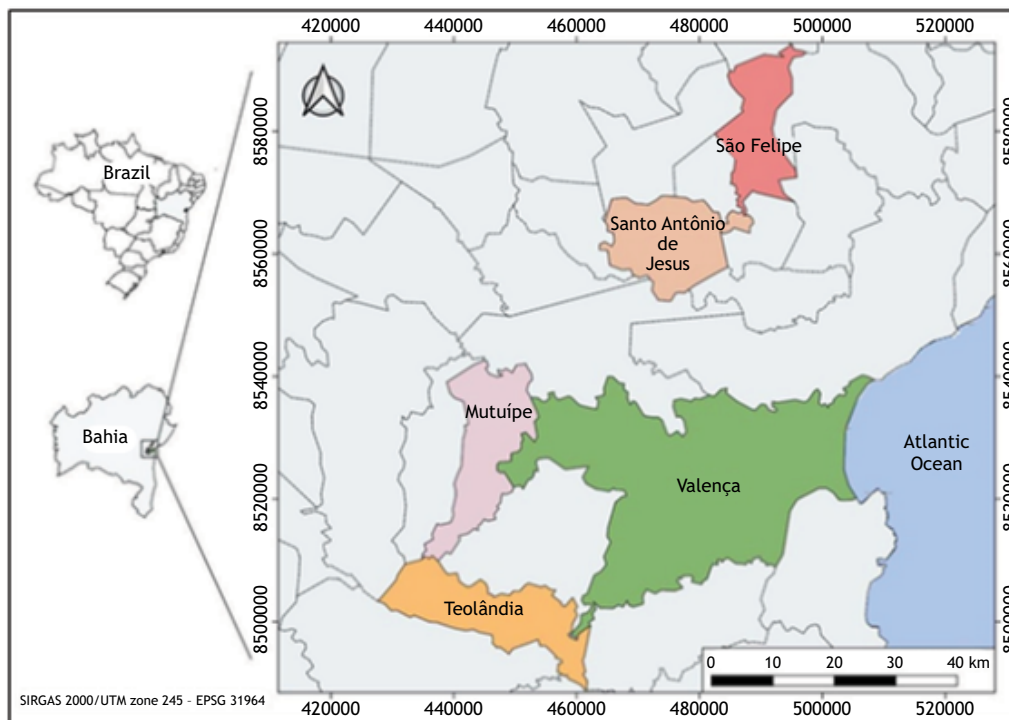
ANVISA's Collegiate Board Resolution (RDC) No. 216 of September 15, 2004<sup>11</sup>, for example, sets out GMP requirements and describes the standard operating procedures that must be adopted by establishments that produce foodstuffs, which includes family agro-industries. This regulation ratifies the obligation to adopt procedures aimed at ensuring the quality of food production. In addition, ANVISA sets the microbiological standards for food produced in Brazil, through RDC No. 724, of July 1, 2022<sup>12</sup>, and Normative Instruction No. 161, of July 1, 2022<sup>13</sup>.

In view of the above, and considering the scarcity of studies analyzing the sanitary conditions of family agro-industries, especially those from EESs, this study aimed to evaluate the microbiological quality of cookies produced by family agro-industries from EESs in Bahia, in order to determine whether the legal requirements are being followed and to contribute to intensifying the adoption of GMPs, thus aiming to promote health.

## METHOD

The study was carried out with 14 batches of cookies, containing 5 samples per batch, totaling 70 samples, as determined by RDC No. 724/2022<sup>12</sup> and ANVISA Normative Instruction No. 161/2022<sup>13</sup>. The microbiological analysis took place from August to October 2022 and the samples came from five family-owned agro-industries in the state of Bahia, located in the municipalities of Santo Antônio de Jesus, São Felipe, Mutuípe, Teolândia, and Valença (Bahia, Brazil), as shown in the Figure.

Around 100 grams of each sample were collected aseptically from the family-owned agro-industries and transported in isothermal packaging to the Microbiology Laboratory of the Multi-disciplinary Health Studies and Research Complex of the Health



Source: Prepared by the authors, 2023.

Figure. Map of Bahia, highlighting the municipalities where microbiological analyses of cookies were carried out in family agro-industries of Solidarity Economic Enterprises in Bahia, Brazil, 2022.

Sciences Centre of the Federal University of Recôncavo da Bahia, where they were immediately analyzed.

Based on the microbiological standard established by RDC No. 724/2022<sup>12</sup> and ANVISA Normative Instruction No. 161/2022<sup>13</sup>, *Salmonella* sp. (AOAC 2014.01) was tested and the quantification of *Escherichia coli* (AOAC 991.14), molds and yeasts (AOAC 997.02), and coagulase-positive staphylococci (AOAC 2003.07) were carried out using the rapid counting method on Petrifilm™ plates (3M Company), as well as the quantification of *Bacillus cereus* (AOAC 980.31), using the traditional method. It should be noted that the quantification of coagulase positive staphylococci was only carried out in the case of cookies with filling<sup>14</sup>.

The plates were read using a manual colony counter (model CP608, Phoenix®) and the results of the microbial counts were expressed in CFU/g food.

The appropriate microbiological standard indicates the acceptability of a batch, for which there is a sampling plan consisting of the number of samples to be analyzed, the maximum acceptable number of sampling units with microorganism counts above the minimum limit (m) and below the maximum limit established (M). In this way, the batch analyzed can be classified as satisfactory with acceptable quality (quantification below or equal to m), satisfactory with intermediate quality (quantification between m and M), and unsatisfactory with unacceptable quality (quantification above M). The microbial population was expressed in

CFU/g and a descriptive analysis of the data was carried out using the Microsoft Excel 2010 program.

## RESULTS AND DISCUSSION

From the data shown in the Table, it can be concluded that 92.9% were of satisfactory quality, of which nine (69.2%) were of satisfactory with acceptable quality and four (30.8%) were of satisfactory with intermediate quality. On the other hand, only one batch (7.1%) was considered unsatisfactory because it had a *Bacillus cereus* count above the limit allowed by current legislation, as guided by RDC No. 724/2022<sup>12</sup> and Normative Instruction No. 161/2022<sup>13</sup>, thus being unfit for consumption.

The results also showed that 100.0% of the samples were free of *Salmonella* sp. and had *Escherichia coli* counts below < 10 CFU/g. The mold and yeast counts were between 10 UFC/g and  $3 \times 10^3$ , with all the samples meeting the standards for these microorganisms. Only one sample of the sequilho *gravata* batch, corresponding to 1.4%, had a coagulase-positive staphylococci count of 10 CFU/g, which is below the value recommended by Normative Instruction No. 161/2022, which sets a maximum limit of  $10^2$  CFU/g<sup>13</sup>.

The quantification of *Bacillus cereus* revealed that one sample from the batch of cheese sequilho had a count of  $2 \times 10^3$  UFC/g, which is above the value also recommended by Normative Instruction No. 161/2022<sup>13</sup>, which stipulates a maximum limit of  $5 \times 10^2$  UFC/g, as well as two samples from the same batch



Table. Results of the microbiological analysis of cookies produced by family agro-industries of Solidarity Economic Enterprises in Bahia, Brazil, 2022.

C	Municipality	Batch (n = 5)	Results (CFU/g)								Conclusion	
			Salmonella	Escherichia coli		Bacillus cereus		Molds and yeasts		Coagulase positive staphylococci		
				Mi	Ma	Mi	Ma	Mi	Ma	Mi		Ma
A	Santo Antônio de Jesus	Coconut sequilho	Absent	< 10	< 10	< 10	< 10	< 10	3 x 10 <sup>3</sup>	*	*	SQI
A	Santo Antônio de Jesus	Cheese sack	Absent	< 10	< 10	< 10	< 10	< 10	< 10	*	*	SQA
A	Santo Antônio de Jesus	Onion sequilho	Absent	< 10	< 10	< 10	< 10	< 10	2 x 10 <sup>1</sup>	*	*	SQA
A	Santo Antônio de Jesus	Sequilho gravata	Absent	< 10	< 10	< 10	< 10	< 10	10 <sup>3</sup>	< 10	10	SQI
B	Mutuípe	Cassava onion sequilho	Absent	< 10	< 10	< 10	< 10	< 10	2 x 10 <sup>1</sup>	*	*	SQA
C	São Felipe	Lemon sequilho	Absent	< 10	< 10	< 10	10	< 10	10	*	*	SQI
C	São Felipe	Corn sequilho	Absent	< 10	< 10	< 10	10	< 10	10 <sup>3</sup>	*	*	SQI
C	São Felipe	Cheese sequilho	Absent	< 10	< 10	< 10	2 x 10 <sup>3</sup>	< 10	10	*	*	INS
C	São Felipe	Passion fruit sequilho	Absent	< 10	< 10	< 10	< 10	< 10	< 10	*	*	SQA
C	São Felipe	Onion sequilho	Absent	< 10	< 10	< 10	< 10	< 10	< 10	*	*	SQA
C	São Felipe	Sequilho goma	Absent	< 10	< 10	< 10	< 10	< 10	< 10	*	*	SQA
D	Teolândia	Coconut sequilho	Absent	< 10	< 10	< 10	< 10	< 10	< 10	*	*	SQA
D	Teolândia	Sequilho with guava filling	Absent	< 10	< 10	< 10	< 10	< 10	10	< 10	< 10	SQA
E	Valença	Onion sequilho	Absent	< 10	< 10	< 10	< 10	< 10	10	*	*	SQA

Source: Prepared by the authors, 2023.

C: Community; n: Number of samples per batch; Ma: Maximum value; Mi: Minimum value; SQI: Satisfactory with intermediate quality; SQA: Satisfactory with acceptable quality; \*: Not evaluated; INS: Unsatisfactory.

with values below the limit recommended by the legislation (7 x 10 UFC/g and 10 UFC/g). Samples from other batches of cookies from the same enterprise, such as lemon sequilho and corn sequilho, showed counts of 10 UFC/g for this microorganism, which is below the maximum limit set by the legislation.

Despite this fact, it should be noted that some samples showed high populations of *B. cereus* and molds and yeasts, which may have been due to a failure in the time and temperature binomial and contaminated raw materials, given that these are more resistant microorganisms.

Molds and sporogenic bacteria are organisms capable of producing spores, however, mold spores are less resistant to heat than bacterial spores<sup>15</sup>. *B. cereus* strains are resistant to various environmental and processing conditions, resulting in food contamination. Although products are subjected to heat treatment, they can remain contaminated. Therefore, incorrect food storage temperature, water activity, pH, and food composition are all factors that contribute to the heat resistance of *Bacillus cereus* during the cooking process, which makes the whole process of food safety and quality much more complex<sup>16</sup>.

In this way, the time x temperature binomial is of great significance when it comes to the microbiological quality of food, directly interfering with the intrinsic contaminating microbiota of the food being prepared. The use of the appropriate

temperature throughout the processing of the food, above 60 °C, can help to guarantee its safety and favor the death of most of the microorganisms that pose a risk to humans, which did not occur with most of the hot preparations from the Food and Nutrition Unit of a University Center in the interior of Ceará<sup>17</sup>.

In the case of *B. cereus*, the high count of this microorganism may have been due to the sporogenic characteristic of the pathogen, which becomes resistant to means of elimination by heat, in addition, the presence of this bacterium has been detected in equipment and utensils, which can be sources of contamination for food, which makes the agent liable to contaminate various stages of food processing, if GMPs are not complied with<sup>18</sup>.

Lentz et al.<sup>19</sup> concluded that *B. cereus* was the main etiological agent involved in food poisoning outbreaks in southern Brazil (2003-2013), with snacks, foods with sauce, and cereals being the main foods involved. In addition, according to Ntuli et al.<sup>20</sup>, cereal grains can be contaminated by pathogenic bacteria throughout the crop's production process, from growth, pre-harvest, post-harvest, drying, transportation, and storage.

Thus, considering that starch is a major ingredient in the production of the sequilhos in question, contamination by *B. cereus* may have occurred right from the cultivation stage. *B. cereus*, which can cause diarrheal syndrome and emetic syndrome, both related to the production of toxins. In diarrheal syndrome, the



toxin is produced after bacterial multiplication in the patient's intestine and in emetic syndrome the toxin is produced before the food is eaten<sup>21</sup>.

In addition to the bacterium *B. cereus*, three samples showed high counts of molds and yeasts ( $\geq 10^3$  CFU/g) in three batches of products. Terrestrial fungi, which include molds and yeasts, can come from the air or soil. They are very adaptable microorganisms and most species are able to use carbon from food as an energy source to stay alive<sup>22</sup>.

However, the identification of possible contaminating fungal species, especially filamentous fungi such as molds, can be a warning about the presence of mycotoxins, which can compromise human health and are cumulative throughout the individual's life. For mold contamination to occur, there needs to be an environment conducive to infection of the food, a factor that can occur at multiple stages, from the planting of grains, preparation of the food, processing, transportation, storage, and distribution<sup>15</sup>. According to Tondo and Bartz<sup>23</sup>, molds have a wide capacity to adapt to different temperatures and this factor can make it difficult to eliminate them. Yeasts, on the other hand, can be harmful and pathogenic. Contamination by these microorganisms can occur at various stages of the production process, generating food losses and significant damage worldwide.

Considering the results found in this research, although most of the plots showed satisfactory results, family agro-industries must produce food safely, given that failures in food processing can lead to contamination by chemical, physical, or biological agents<sup>24</sup>. Another factor is the importance of food production for those involved. In recent years, family farming has emerged as a resistance to the technological, organizational, and modernization development processes of Brazilian agriculture and the growth of large commodity-producing estates destined for export, to which a large part of government capital is allocated. Due to the impacts of the social vulnerability generated in Brazil and other countries, family agro-businesses aim to diversify their production in order to increase profitability and circumvent the difficulties imposed<sup>25</sup>.

This research sought to evaluate the microbiological quality and reiterate the cultural importance of the food produced by family agro-industries, traditionally known as "sequilhos", made with ingredients that are culturally used in the region, such as cassava starch, passion fruit, corn starch, fresh coconut, guava, and cornmeal. By selling these products, the producers see the possibility of earning money.

The inclusion of EES agro-industries in the local consumer market helps to increase the profitability and value of products from family agro-industries. This validation can also take place by proving the good hygienic and sanitary quality of the foodstuffs, thus enabling greater acceptance of these foods by buyers and commercial establishments, which are increasingly concerned about the condition of what is being offered.

To this end, ANVISA establishes as one of the principles for this segment the harmonization of procedures to promote the formalization and health safety of products and services provided by rural family enterprises and EES, considering customs, traditional knowledge, and applying the good practices established by the Health Surveillance (VISA) bodies<sup>26</sup>.

In order to monitor compliance with the law, there is VISA, which is a public body responsible for supervising and ensuring that products that reach consumers do not compromise their health. ANVISA therefore approved RDC No. 216/2004 with the aim of standardizing sanitary control actions in the area of food. This regulation contains at its core "Good Practice Techniques for Food Services" which applies to food services that carry out activities involving handling, preparation, fractioning, storage, distribution, transportation, display for sale, and delivery of prepared food for consumption<sup>11</sup>.

These health measures are regulated by ANVISA's RDC No. 49 of October 31, 2013, which "provides for the regularization of individual micro-entrepreneurs, rural family enterprises, and EES to carry out activities of health interest."<sup>24</sup>.

In addition, it is of the utmost importance that all establishments that produce and sell food are aware of and comply with the provisions of RDC No. 216/2004<sup>11</sup>, regarding GMP in food services, such as the family agro-industries of EES. Compliance with this Resolution reduces failures and waste during food preparation, positively raises the standard of food handling and hygiene of the environment, utensils, and handlers, in addition to standardizing and qualifying production processes, enhancing the image of the agro-industry in the market, favoring profitability, increasing credibility, and corroborating the reduction in the occurrence of FBD outbreaks due to the supply of safe food.

Thus, according to Shinohara et al.<sup>27</sup>, health inspection is a protection procedure that includes the producer as well as the consumer, with the aim of certifying good manufacturing conditions that fulfill the purpose of feeding and nourishing. Therefore, it is worth highlighting the important role that the Good Manufacturing Practices Manual and the Standard Operating Procedures play within an enterprise, guiding them in accordance with RDC No. 216/2004<sup>11</sup>, in order to standardize and guarantee the sanitary quality of food, adapting the process of manufacturing, sanitizing, handling, and storing food, in accordance with the technical regulations.

That said, the majority of cases of FBD stem from inadequate conduct throughout the production process and behaviors that favor contamination by microorganisms<sup>8</sup>, especially bacteria and fungi, and it should be noted that handlers can also be significant vectors along the way. Thus, having processes that aim to adapt and standardize production, in addition to meeting the requirements of ANVISA and current legislation, provides sanitary quality, generating value for local culture and commerce, reducing losses for producers, and resulting in greater profitability. To this end, the microbiological analysis of the sequilhos produced allows for the early detection of foods containing the





pathogens mentioned above, guaranteeing the sanitary quality of the sequilhos produced by EES family agro-industries and the safety of consumers<sup>28</sup>.

## CONCLUSIONS

Considering that 13 of the 14 batches evaluated were of satisfactory quality, the cookies manufactured by family agro-industries

in the EES of Bahia were low in contamination and did not represent a risk for the transmission of FBD, making them suitable for human consumption.

However, given the importance of cookie production for the communities involved, it is suggested that public policies be drawn up that involve health education for handlers, increasing production and valuing regional culture.

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#### Authors' Contribution

Santos SEF, Freitas F, Silva IMM - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the paper. Jesus DS - Acquisition, analysis, data interpretation, and writing of the paper. Silva JN - Analysis and writing of the paper. Camilo VMA - Conception, planning (study design), data interpretation, and writing the paper. Velloso TR - Conception, planning (study design), and writing of the paper. All the authors approved the final version of the paper.

#### Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.



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