## Microbiological quality of powdered shrimp

## Qualidade microbiológica de camarão em pó

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## ABCTRACT

In order to analyze the microbiological quality of shrimp powder marketed in municipalities in southwestern Bahia, 15 samples from three distinct brands were collected and microbiological analyses were performed for *Salmonella* spp., coliforms and coagulase-positive *Staphylococcus*. All samples analyzed were contaminated with total coliforms and thermotolerant, with counts above  $10^2$  CFU.g<sup>-1</sup>. Only sample C1 was contaminated with coagulase-positive *Staphylococcus*, presenting values of 4.60 x  $10^5$  UFC.g<sup>-1</sup>, above the maximum value (5 x  $10^2$  CFU.g<sup>-1</sup>), tolerated by Brazilian food safety standards. In qualitative tests for *Salmonella* spp., all samples were positive for this pathogenic bacterium. Given the results found, it was observed that powdered shrimps marketed in the municipalities of southwestern Bahia have low quality and unsatisfactory hygienic-sanitary conditions, and consumer health can be considered at risk.

Key words: Salmonella spp., coagulase-positive Staphylococcus, coliforms.

## RESUMO

Com o objetivo de analisar a qualidade microbiológica de camarão em pó comercializado em municípios do sudoeste da Bahia, foram coletadas 15 amostras de três marcas distintas e realizadas análises microbiológicas para *Salmonella spp.*, coliformes e *Staphylococcus* coagulase positiva. Todas as amostras analisadas estavam contaminadas com coliformes totais e termotolerantes, com contagens acima de 10<sup>2</sup> UFC.g<sup>-1</sup>. Apenas a amostra C1 estava contaminada com *Staphylococcus* coagulase positiva, apresentando valores de 4,60 x 10<sup>5</sup> UFC.g<sup>-1</sup>, estando acima do valor 5 x 10<sup>2</sup> UFC.g<sup>-1</sup>, tolerado pela legislação brasileira. Para a pesquisa de *Salmonella spp.* todas as amostras apresentaram presença dessa bactéria patogênica. Diante dos resultados encontrados, observou-se que os camarões em pó comercializados nos municípios do sudoeste da Bahia apresentam condições higiênico-sanitárias insatisfatórias e baixa qualidade, podendo ser considerados um risco a saúde do consumidor.

Palavras-chave: Salmonella spp., Staphylococcus coagulase positiva, coliformes.

## **1 INTRODUCTION**

The technique of shrimp production has been practiced in Brazil for more than 30 years and it is considered a sustainable activity. Besides, it contributes to the surrounding community since it generates jobs and income to all those involved in the production chain. Among the main advantages for producers investing in shrimp are: short duration of crops, high prices of the product in the market and favorable climatic conditions in Brazil for cultivation (ENGEPESCA, 2019). It is added that high demand for healthy products with high nutritional value has further leveraged the national and international markets to increase crustacean consumption.

Shrimp is a crustacean widely consumed in Brazil and worldwide. According to FAO, 125,347 tons of crustaceans were consumed in Brazil in 2013, with shrimp, worldwide, the second most commercially important product (FAO, 2013; 2018) Shrimp has in its composition a great amount of proteins, a factor that contributes to the increase in its demand for consumers, especially those who seek a healthier diet. In addition to nutritional composition, this product is considered an indispensable and characteristic ingredient of several typical Brazilian dishes, especially in the northeast region.

Shrimp is a highly perishable food and requires a method of conservation that prolongs its commercial life. In regard to food preservation, dehydration is one of the most used methods because it prevents deterioration by microorganisms and chemical reactions, in addition to adding monetary value to the product due to the applied processing method (COSTA et al., 2019; TOPUZ et al., 2017).

In the Brazilian state of Bahia, regional gastronomy was built under strong influences of indigenous, Portuguese and African cultures, making recorded its customs and inheritances of products in Bahian culture such as palm oil, coconut milk, fish and pepper. Whether in the capital or

in surrounding cities in state of Bahia, it is common to find traditional tents or establishments that sell typical dishes such as *acarajé*, *abará*, *vatapá* and *caruru* throughout the year, and a considerable increase in the consumption of these foods in the period of "Holy Week" (VIEIRA, 2004; CANTARINO, 2006).

All these typical preparations use powdered shrimp as its main ingredient, a product obtained through salting, drying and crushing shrimp. These stages of production should be performed considering good manufacturing practices, so that there are no physical and also biological contaminations by bacteria, such as coliforms, *Staphylococcus* and *Salmonella*, which cause disorders to human health and, in some cases, also lead to death.

Among the various factors that contribute to the occurrence of food diseases, mainly, flaws in hygienic-sanitary control and inadequate processes and storage of the final product (FORSYTHE, 2013) stand out. In Brazil, inspection service is divided into: Municipal Inspection Service (S.I.M.), State Inspection Service (S.I.E.) and Federal Inspection Service (S.I.F.) and Brazilian System for The Inspection of Products of Animal Origin (SISBI). All establishments need to be registered with the competent organization for the supervision of their activities (SALAZA, 2018).

Therefore, the aim of this study was to evaluate the microbiological quality of powdered shrimp marketed in different establishments in the municipalities of southwest Bahia, Brazil.

### 2 MATERIALS AND METHODS

#### **Sample collection**

The powdered shrimp samples were acquired in two cities in the southwestern region of the state of Bahia, Brazil, totaling 15 samples from 3 different brands, two industrialized and one sold in bulk. For each brand, 5 samples were selected, all belonging to the same batch. After collection, the powdered shrimp samples were forwarded to the Food Microbiology Laboratory of the State University of Southwest Bahia (UESB), Itapetinga, Bahia, Brazil, in which the following microbiological analyses were performed: total and thermotolerant coliforms, *Salmonella* spp. and coagulase-positive *Staphylococci*.

### **Microbiology tests**

For the analysis, 25g of each sample was weighed and the dilution was performed in 225 mL of peptone water (dilution 10<sup>-1</sup>) until dilution 10<sup>-5</sup>. 1.0 mL aliquots of each dilution were inoculated in duplicate in MacConkey Agar medium, with incubation at 36 °C/30-48h for the determination of coagulase-negative *Staphylococci*. After this period, the typical colonies of *Staphylococcus* found in

Petri dishes were stained using Gram technique, in order to confirm the presence of Gram-positive cocci (CERQUEIRA, 2013).

In order to perform the tests for determination of coliforms, 1.0 mL of each dilution were inoculated in Violet Red Bile Agar (VRBA) medium, with incubation at 36 °C/18-24h. Then, the visualization of results was performed to determine the probable amount of bacterial population. Positive samples were also submitted to the confirmatory test for total coliforms (incubation in brilliant green bile broth with 2% lactose at temperature of 36 °C/24-48h) and thermotolerant coliforms (incubation in EC broth, at temperature of 45 °C/24-48h in water bath, under agitation), and positive samples that presented gas formation in the Durhan tube (CERQUEIRA, 2013).

Simultaneously, tests for the presence of *Salmonella* spp. was performed by incubation of the dilutions 10<sup>-1</sup> of each sample per 18-24h, at 36 °C. After this period, 1.0 mL from each sample were collected to perform the selective enrichment growth in tubes containing 9.0 mL of Rappaport Vassiliadis broth and in tubes containing 9.0 mL of Selenito-Cystine broth, being incubated by 24h/36°C. After the selective enrichment growth phase, samples of each culture medium were inoculated, in duplicate, in plates containing Xylose Lysine Deoxycholate Agar (XLD Agar) and Bismuth Sulfite Agar (BSA). Finally, samples were incubated by 24h at 36 °C.

### **3 RESULTS AND DISCUSSION**

According to results, all 15 samples of the 3 brands analyzed presented contamination by both total and thermotolerant coliforms (Table 1) according to tests, with a population above 10<sup>2</sup> CFU.g<sup>-1</sup>, which represents the maximum limit established by Brazilian Legislation (BRASIL, 2001). This indication showed that even salty and dry fish, with high sodium chloride content, was not exempt from chemical or microbiological deterioration (JAY, 2005). Thus, it was possible to infer the inefficiency in the processing of salting and drying in the samples of shrimp powder, because according to Niamnuy, Devahastin and Soponronnarit (2007), the correct heating process, for 3 minutes of the shrimp in a 4% NaCl solution is sufficient to reduce the microbial population to safer standards. In addition, the occurrence of coliforms in food deserves to be highlighted, since it is still related to the hygienic-sanitary quality of the product.

In contrast, it is possible to find discrepant results in literature to those performed in this study, such as observed by Lourenço et al. (2011), in which coliforms resistant to 45 °C were detected in populations lower than  $1.0 \ge 10^1$  CFU/g in salted Piracuí fish; and Nunes et al. (2012), reporting only 0.3 MPN/g of bacterial population in dry Aviú shrimp samples commercialized at street markets in the city of Belém, Pará, Brazil. Evangelista-Barreto et al. (2016) checked the salty, dry and/or smoked

shrimp marketed at the street markets in Cruz das Almas, Bahia, Brazil, exhibiting coliform counts of <3.0 NMP/g, in accordance for consumption.

However, Sereno et al. (2011) analyzing *acarajé* samples and its complements, recorded nonconforming samples for coliforms at 45 °C in *vatapá* (26.7%) and shrimp samples (30%), indicating post-processing recontamination since the products are previously submitted to heating process.

**Table 1.** Bacterial counting for coliforms, coagulase-positive *Staphylococci* and *Salmonella* sp. in powdered shrimp samples, marketed in the municipalities of Southwest of Bahia, Brazil.

Brands	Analysis		
	Coliforms (CFU.g <sup>-1</sup> ) *	Coagulase-positive Staphylococci	Salmonella spp.
C1	7,27 x 10 <sup>4</sup>	$4,60 \ge 10^5$	Presence
C2	$2,25 \ge 10^5$	Absence	Presence
C3	3,90 x 10 <sup>5</sup>	Absence	Presence

\* CFU.g<sup>-1</sup>= colony forming unit by gram.

Regarding the test of *c*oagulase-positive *Staphylococci* counting, only in the C1 brand exhibited a significative population of  $4.60 \times 10^5$  CFU.g<sup>-1</sup>, which represents a value above the limit  $(5 \times 10^2 \text{ CFU.g}^{-1})$  of the Technical Regulation on Microbiological Standards for Food<sup>7</sup>. This level of high contamination may be associated with inadequate manipulation during the processing of powdered shrimp by the handlers as well as the lack of hygiene of the utensils used, which may have favored cross-contamination by these microorganisms. Lira et al. (2013), determine that the maximum concentrations of enterotoxigenic *Staphylococcus* sp. are  $10^5$  CFU.g<sup>-1</sup>.

Nunes et al. (2013), performing the coagulase-positive *Staphylococci* detection in samples of dried Aviú shrimp and Piracuí fish, observed unusual growth ranging from 1.0 x  $10^3$  to 1.66 x  $10^7$  CFU.g<sup>-1</sup>. Lira et al. (2013), found the value of 4.5 x  $10^5$  UFC.g<sup>-1</sup> when evaluating smoked shrimp from Pontal do Peba region, Alagoas, Brazil. Evangelista-Barreto et al. (2016), verified *Staphylococcus* sp. with values from 1.0 x  $10^1$  to 9.9 x  $10^6$  UFC.g<sup>-1</sup>, however, when evaluating coagulase-positive *Staphylococci* were in agreement with the legislation.

Therefore, *Staphylococci* in high densities in food pose a risk to human health, because of their potential in the production of staphylococcal enterotoxin. It is a major concern the existence of samples inappropriate for consumption, since the higher the bacterial population, the greater the probability of producing toxins and, consequently, the risk of food poisoning due to the intake of these products, even when submitted to the cooking or frying process (SILVA JÚNIOR, 2013)

It is noteworthy that in brands C2 and C3 the result was the absence of coagulase-positive *Staphylococci*, but it was observed the positive result for presence of bacterial groups such as Gram-

positive bacilli and *Enterococcus* spp. According to Franco and Landgraf (2008), *Enterococci* are resistant to adverse environmental conditions and survive in dried and cured foods. This report was proven by Nunes et al. (2012), when they detected this bacterium in dry salted pirarucu.

For the qualitative analysis of *Salmonella* sp., all samples exhibited positive results for the presence of the microorganism, demonstrating the insecurity in the safety of food since it should be absent in 25g of food samples (BRASIL, 2001). This result was similar to that found in studies conducted by Evangelista-Barreto et al. (2016) confirming the presence of *Salmonella* sp. populations in 5% of dry shrimp samples; and Nunes et al. (2013) who detected this pathogen in three samples of salted Aviú shrimp samples.

The presence of this microorganism in food has a great impact on public health, due to high endemicity, high mortality rate and, above all, the difficulty of control, representing a significant cost to societies in many countries (WAN-NORHANA et al., 2018).

## **4 CONCLUSION**

Given the results found in the present study, it is possible to affirm that the brands of powdered dry shrimp marketed in the cities of Southwest Bahia have low microbiological quality, being produced and marketed in facilities under unsatisfactory hygienic-sanitary conditions.

Thus, it is suggested the need for control actions by health surveillance authorities in order to ensure the marketing of a product within microbiological legal standards in order to avoid risks to consumers health.

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