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RESEARCH ARTICLE OPEN ACCESS

BACTERIOLOGICAL AND PHYSICAL-CHEMICAL EVALUATION OF OPEN CANNED SARDINES PRESERVED IN REFRIGERATION

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ABSTRACT

Consumers are becoming increasingly conscious about the food they buy, and with this comes the necessity for more information about food quality and safety. In addition, conscious consumption and, consequently, less waste generation is also becoming a concern.To verify the efficiency of the commercial sterilization process and the safety of the food for a period longer than indicated by manufacturer, during one week, bacteriological and physical-chemistry analyses were performed on samples of canned sardines in different coverage obtained in commercial establishments in the state of Rio de Janeiro. Microbiological analyses consisted of mesophilic aerobic microorganism count (MC), psychrotrophic aerobic microorganism count (PC), thermophilic aerobic microorganism count (TC), anaerobic sulphite reduction at 46 ºC count (ASR), *Bacillus* spp. count, histamine-forming bacteria count (HBC) and sporulated bacteria count (SC). The physical-chemical analyses included the determination of hydrogen potential (pH) and the detection of histamine by spectrofluorimetry. There were no samples with values above 100 mg/kg of histamine and association with the HBC. No counts above 10^5 were observed in any analysis on all days. The presence of *Bacillus* spp. and spores were within the limits established by legislation,thus the food remained safe until the seventh day of storage.

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INTRODUCTION

In nutritional point, the fish is a major source of protein abundant in minerals including calcium, iron, zinc, selenium and iodine, vitamin D and long-chain omega 3 poly-unsaturated fatty acids. The adequate consumption is associated with lower risks of development of heart diseases, stroke and Alzheimer disease (Jinadassa et al., 2020).

Other characteristics like highly digestibility proteins and elevated water activity turn fish a fast-deteriorating product and demand industries to research for new alternatives to delay this process. One of the options found was the production of canned fish. In the process of producing canned products, the food in the can undergoes a heat treatment know as commercial sterilization that ensure consumer safety. Elements related to acceptable organoleptic quality, commercial viability and conditions that may occur inside or outside the can are relevant in guaranteeing the effectiveness of commercial sterilization (Shaw, 2010).

The canned sardine is classified in accordance with the pH as low-acid food (above 4.6), a range where *Clostridium botulinum* can develop and produce toxins.

Moreover, it is a microorganism of importance in public health, consequently in commercial heat sterilization, the destruction of this bacteria is the main objective of this thermal processing. Due to the type of thermal processing applied to canned food, microbial deterioration should not occur, however, problems in the production flowchart of these products, like deterioration of raw material, contamination caused by failures in the interlocking stage, under processing because of lower temperatures and inadequate cooling may cause microbiological change in the final product (Silva et al., 2017; Berk, 2018).

Which in comparison to fresh fish, has the advantages of a longer commercial shelf-life, storage without refrigeration, low cost and no need for food preparation. These aspects associated to their nutritional value made this product be recommended by World

Health Organization (WHO) during the COVID-19 pandemic (FAO, 2020a; WHO, 2020).

According to FAO (2020b), 35% of entire global harvest from fisheries and aquaculture are wasted annually and that improvements in services and structures would case less pressure on fish stocks and enhance resource sustainability and food security. The increase in fish stocks is especially important in the case of sardines (*Sardinella* spp.) which are being overfished in some areas of the world.

Defined as a decrease in quantity and/or quality of food, through the actions of food service sectors, retailers and households, food waste has become a major concern for sustainability policy and food industry, being estimated 60 kg/capita in Brazilian residences (Porcino et al, 2018). The consequences of food waste cause negative impacts on environment, where it is estimated that about 8-10% of greenhouse gas emissions come from unconsumed food, and economically with the overload on the waste management system (United Nations Environment Programme, 2021). Consumer- related factors such as excessive amount of prepared food, non-reuse leftovers and disposal products close to expiration date are most associated with waste. (Romani et al., 2017). In this context, the aim of the present study was to evaluate the bacteriological and physical-chemical quality of opening canned sardines preserved in refrigerator.

MATERIALS AND METHODS

The twelve sardine (125 g) cans were collected in supermarkets in the city of Rio de Janeiro after visual inspection, selecting those that had not been dented, stewed and rusted. For each pool sample, it was necessary four cans with the same brand, lot and flavor. In total six brands were analyzed in tomato sauce and oil flavors.

The samples were transported at room temperature. In the laboratory, cans were externally sanitized with alcohol 70 % and opened with sterilized instruments. The contents of each can was poured into a sterile bag and homogenized with the aid of the Stomacher (Seward Stomacher 80®, United Kingdom) for two minutes. Subsequently, 25 g of sample pool was withdrawn for each day for microbiological analyses, 10 g for pH measurement and 5 g for histamine detection. All samples were stored in refrigerator (4 °C). The day of analyses were: day 0 (after opening the can), day 1 (24 hours after opening), day 2 (48 hours after opening) and day 7 (168 hours after opening).
The microbiological analyses included a mesophilic aerobic microorganism count (MC) (Ryser et al., 2015), psychrotrophic aerobic microorganism count (PC) (Vasavada et al., 2015), thermophilic aerobic microorganism count (TC) (Jackson, 2015), anaerobic sulphite reduction at 46 ºC count (ASR) (Labbe, 2015), Bacillus spp. count (Benetti et al., 2015), histamine-forming bacteria count (HBC) (Pan et al., 1985) and sporulated bacteria count (SC) (Brazil, 1981). All microbiological analyses were performed in duplicate, to increase the representativity up to 10-2 dilution providing greater chances of positivity because they were products submitted to commercial sterilization.

The physical-chemical analyses included determination of pH (Institute Adolf Lutz, 2008) and histamine detection by spectroflourimetry (AOAC, 2000). For pH measurement, the samples were read by the peagometer (Digimed® DM-22) in triplicate and the result was based on arithmetic mean of measurements. The histamine detection was read in duplicate. Statistical analysis was performed by SPSS version 18 through exploratory analyses and non-parametric tests of Kruskal-Wallis, Mann-Whitney, Friedman and Wilcoxon.

RESULTS AND DISCUSSION

pH and Histamine: As for pH the results were found for tomato sauce was 5.48±0.13 for day 0; 5.45±0.14 for day 1; 5.49±0.16 for day 2 and 5.51±0.13 for day 7. For oil the results were 5.75±0.27 for day 0; 5.76±0.27 for day 1; 5.79±0.36 for day 2 and 5.80±0.34 for day 7. The covering medium influenced the pH of the sample, although there was only significant difference on day 1 ($p<0.05$). In comparing brands, the sample sardine with oil of mark C had higher values compared to the other brands and obtained the greatest difference between days 1 and 2 of storage. The brand D in oil was the most constant pH throughout the days (Fig. 1). In a study carried out by Schmidt (2014) in which the pH of two commercial brands, denominated A and B, of sardines in oil was evaluated, average was 5.96 ± 0.09 and 5.94 ± 0.01. These results are smaller than majority brands except the brand "C" considering only the opening day of the can (day 0).

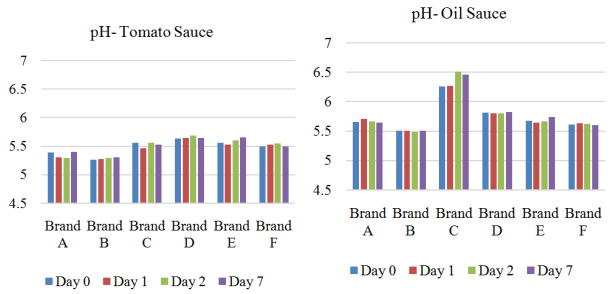

Figure 1. Distribution of pH in different brands

Pereira (2014) in a similar study, also found even greater results for canned sardines in oil: 6.64 ± 0.04 for day 0; 6.56 ± 0.04 for day 1; 6.51 ± 0.04 for day 2 and 6.52 ± 0.08 for day 7. When compared to canned sardines with tomato sauce, the results were also higher: 5.59 ± 0.05 for the day 0; 5.93 ± 0.07 for day 1; 5.89 ± 0.03 for day 2 and 5.90 ± 0.08 for day 7.

The pH level changes according to the time of death, ranging from near neutrality when alive to 6.0-6.8 immediately after death due to conversation of glycogen to lactic acid. The maintenance of the pH in this range may be justified by absence of bacterial activity that produces alkaline compounds and raises the pH (Monteiro et al., 2018).

Considering that there is no pH standard for canned sardine in Brazilian and international legislation, it concluded that all sample, were fit for consumption supported by the legislation for fresh fish where the value is recommended below 7.0 (Brazil, 2017).

The histamine levels were below the detection limit (≤0.13) in all samples on days 0 and 1. The value of histamine in canned sardine with tomato sauce were ≤0.13 for day 0 and 1; 1.47±0.25 for day 2 and 4.19±1.16 for day 7 and with oil were ≤0.13 for day 0 and 1.44±0.16 for day 2 and 6.59±1.37 for day 7. There was no difference in pH between the means of covering the canned sardines on days 0, 1 and 2. On day 7 there were signs of significant difference ($p<0.05$). When comparing the brands, the sample of sardines with tomato sauce of brand C showed the smallest difference between the days. In contrast, the sardine sample with oil from brand B indicated the greatest difference between the days of storage (Fig. 2).

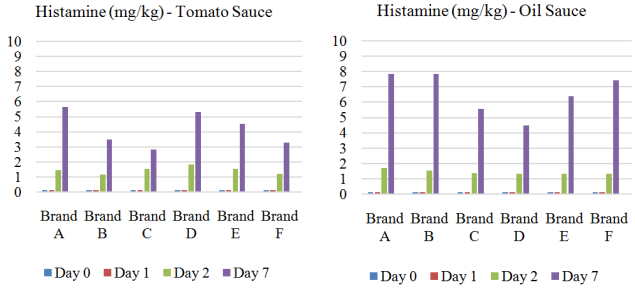

Figure 2. Distribution of histamine in different brands

In the study carried out by Pereira (2014), higher values were observed for the preservation of sardines in oil for days 0 (1.5±0.4) and 1 (1.7±0.2). For the second day of storage, the numbers were

close (1.4±0.2), but for the last day the result was lower than the present study (2.5±0.3). In the comparison of sardines in tomato sauce, the values were higher on days 0 (1.7±0.2), 1 (1.6±0.1) and 2 (1.7±0.2) days and lower on day 7 (3.1±0.1).

An important factor that influences aminodecarboxylase activity is the pH level, where it produces most in a medium with an optimum pH of 4.0-5.5 (Bilgin et al., 2015). Therefore, it is deduced that the pH of the medium in the sardines was one of the factors responsible for the low enzymatic activity. Combined with good hygiene conditions and maintaining the temperature of the raw material below 4°C (FDA, 2020).

Based on all the results obtained, all the samples conformed to the requirement determined by standard of canned sardines not exceeding 100 mg/kg (Commission Regulation EC 2073, 2005).

Microbiological analyzes: The results of microbiological analyzes are shown in Tab. 1.

There was no growth of ASR at 46°C (<10 CFU est) in any sample. The analyzes of MC, TC and PC were used to evaluate the quality of the sample. The lower count, even in the individual counts by brands (Fig. 3), could be justified by the good hygiene conditions adopted and the adequate storage temperature despite the period exceeding the expiration date.

Table 1. Microbiological counts during storage days

Analyses	Day 0	Day 1	Day 2	Day 7
	CANNED SARDINE – TOMATO SAUCE (CFU/g)			
MC	$1.8x10^2$	$4.2x10^1$	$1.1x10^2$	$1.5x10^2$
TC	<10 est	$1.7x10^2$	$2.6x10^2$	$2.5x10^2$
PC	<10 est	$1.7x10^2$	$2.5x10^2$	$8.3x10^2$
SC	$5.0x10^2$	$8.3x10^2$	$6.1x10^3$	$2.4x10^3$
HBC	$1.4x10^2$	$0.8x10^1$	$1.1x10^2$	$3x10^1$
ASR	<10 est	<10 est	<10 est	<10 est
Bacillus spp.	$0.8x10^1$	$0.7x10^1$	$0.2x10^1$	<10 est
	CANNED SARDINE – OIL (CFU/g)			
MC	$9.2x10^1$	$1.2x10^2$	$9.2x10^1$	$9.2x10^1$
TC	$6.7x10^2$	$2.5x10^2$	$1.2x10^3$	$4.2x10^2$
PC	$8.3x10^1$	$8.3x10^1$	<10 est	$7.5x10^2$
SC	$7.7x10^3$	$8.5x10^2$	$1.4x10^4$	$3.7x10^3$
HBC	$7.5x10^1$	$1.7x10^1$	$5.0x10^1$	$2.5x10^1$
ASR	<10 est	<10 est	<10 est	<10 est
Bacillus spp.	$0.7x10^1$	<10 est	$0.3x10^1$	$0.2x10^1$

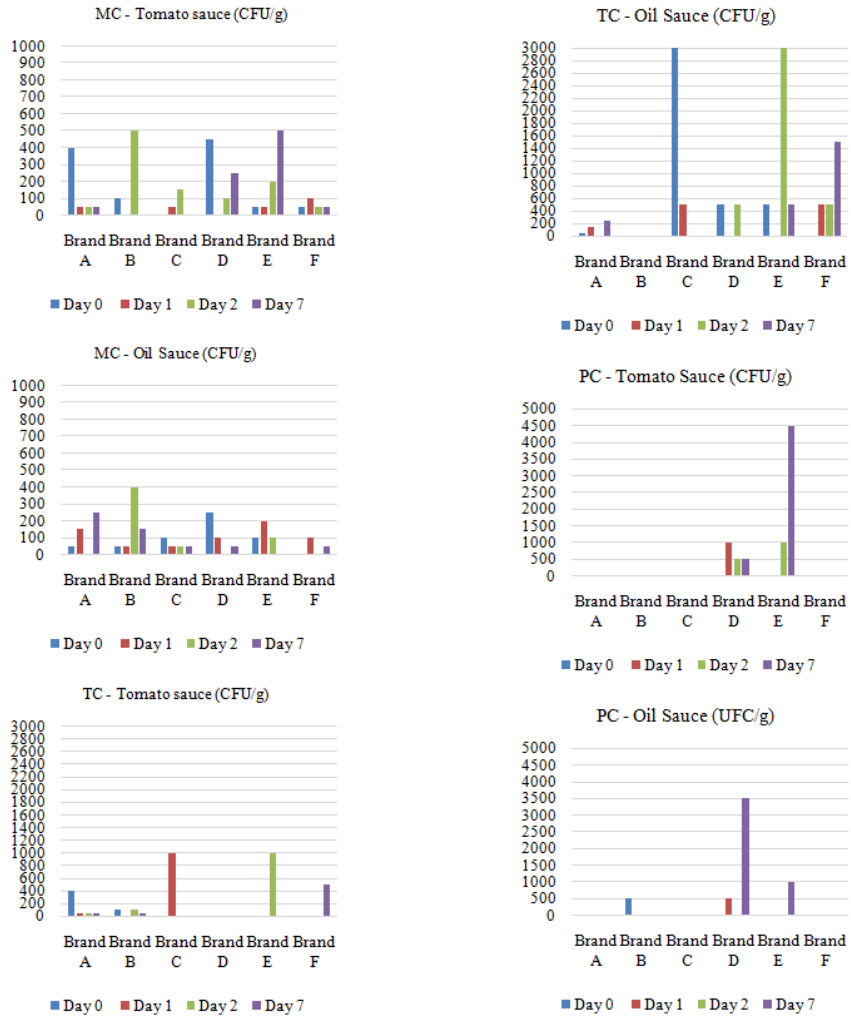

Figure 3. Distribution of MC, TC and PC in different brands

Similarly, to the study developed by Pereira (2014). In compliance with the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), counts below 10^5 for fish are considered satisfactory.

Although the sterilization conditions used in the study do not portray the reality of the households, Brazilian homes are the places where 37% of the diseases transmitted by food occur (SINAN, 2019), this percentage is lower compared to the United States (only 8%) (CDC, 2019).

The SC was higher when compared to the other analyzes, however the commercial sterilization procedure does not the product sterile, because the minimum acceptable level to ensure sterilization is from a non-sterile unit to 10^{-6} (Dunkelberg et al., 2009). Also, even at low concentration, highly resistant spores appear in the raw material and on the production line (André et al., 2013). There was a lot of discrepancy between the brands and between the means of coverage, an example is the D brand where in the tomato sauce it had the highest consistency, but in the oil sauce it obtained the highest count on days 1 and 2 compared to all other samples. (Fig. 4).

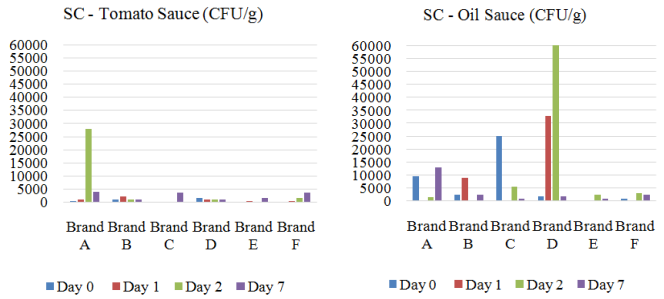

Figure 4. Distribution of SC in different brands

The HBC was performed for the purpose of evaluating and comparing the bacterial count with the histamine content detected by the spectrophotometer. It was deduced that there was a low relation between the count and the value quantified by physical-chemical method, since counts were higher than 10^2 CFU/g in the first two days of analyses and greater than 10^1 CFU/g in the third day (Fig. 5), however, there were only values different from zero in the spectrophotometer from day 2 of storage.

One possible reason for this occurrence is that the histamine-forming microorganisms such as *Hafnia alvei*, *Morganella morganii*, *Morganellapsychrotolerans*, *Photobacterium phosphoreum* and *Klebsiella pneumoniae* are inhibited according to the pH of the medium. However, at low pH, these bacteria are stimulated to produce decarboxylase as a mechanism against the acidity of the medium. The final result depends on the balance of these opposing factors (EFSA, 2011). In this context, it is assumed that the pH in the first two days was not low enough to stimulate decarboxylase production but allowed bacterial growth due to the low acidity of the food.

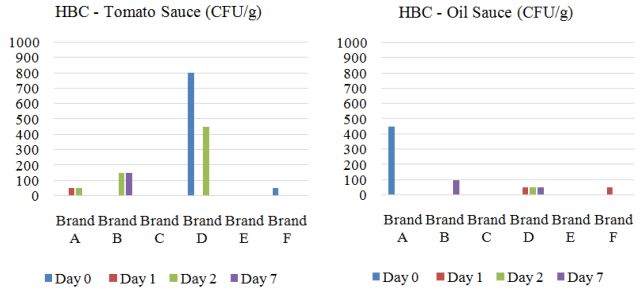

Figure 5. Distribution of HBC in different brands

Another important result was the identification of *Bacillus* spp. with typical colonies that in the bacterioscopy presented Gram-positive rods with large number of spores. Just the F mark did not contain *Bacillus* spp. in both coverages. (Fig. 6).

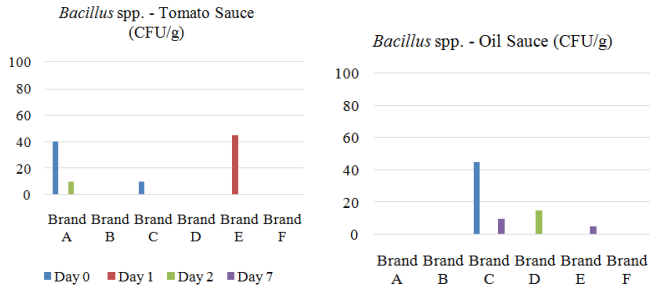

Figure 7. Distribution of *Bacillus* spp. in different brands

The high count of bacterial spores and the presence of *Bacillus* spp. in the work can be according to Ye et al. (2019) where the genus of *Bacillus* and *Acinetobacter* were predominant in 100% and 87.5%, respectively, of the domestic refrigerators. Nevertheless, a *Bacillus* spp. count under 10^3 was expected within a satisfactory result as per FSAI (2001). As for the days of storage, it was found that there was no constant growth of microorganisms over the days. This fact could be justified by the different growth phases encountered by the bacteria. That means, the highest count in the first days and subsequent decrease of some microorganisms is based on rapid multiplication when reaching the log phase slightly and then the decline phase. In turn, the highest count in the last days is supported by slow growth until it reaches the log stage. Considering the results found in the physical-chemical as well as in the microbiological analyses, it suggested that the samples were suitable for consumption up to seven days of storage.

CONCLUSION

The term commercial sterilization occurs due to the high temperatures in which the food is submitted. However, a minimum count can be remaining without compromising food safety. Although factors such as the coverage medium and the canned sardine brand influenced the results presented, all obtained satisfactory results. The maintenance of food safety of canned sardines for a longer period than that determined on the packaging, guarantees the health of its consumers and allows the least waste of food. In the search for less food waste, behavior changes regarding food are necessary such as reusing leftovers, planning meals e not buying on impulse only considering the promotional price.

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