Hygienic and sanitary characteristics of dry salted fish marketed in Porto Velho city, Rondônia – Brazil

Características higiênico-sanitárias de pescado salgado seco comercializado em Porto Velho, Rondônia – Brasil

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ABSTRACT: Handcrafted salted fish is marketed weekly in retail markets and public fairs in the Porto Velho city, Rondônia state, Brazil. Knowing the microbiological quality of these products is essential for public health, given that such products are not subject to any quality control. The aimed of the study was to evaluate the hygienic-sanitary characteristics and the moisture content of pirarucu and salted and dried shrimp marketed in the Porto Velho city. Twenty samples were collected, 10 pirarucu and 10 shrimp, in February and June 2019. For microbiological analysis, surface plating was performed using acidified potato agar, for molds and yeasts, and PCA agar for halophilic bacterias. Moisture was determined by gravimetry, using an oven at 105°C. Results for molds and yeasts on pirarucu ranged <10 log CFU/g (absent) to 3 log CFU/g. For shrimp, values ranged <10 log CFU/g to 3.57 log CFU/g. For halophilic bacterias, contents ranged <10 log CFU/g to 6.30 log CFU/g in pirarucu samples and <10 log CFU/g to 6.97 log CFU/g in shrimp samples. The variation in moisture content ranged 36.99 to 54.31% for pirarucu, and 40.6 to 56.82% for shrimp. The results obtained may be related to poor hygienic conditions in processing, the lack of hygiene of utensils and places for handling, or even the quality of the raw material used.

KEYWORDS: Halophilic bacterias; molds and yeasts; shrimp; pirarucu.

INTRODUCTION
Brazilian agribusiness boosts the recovery of the national Gross Domestic Product (GDP). According to surveys by the Centro de Estudos Avançados em Economia Aplicada, Universidade de São Paulo (CEPEA/USP), even amid the national crisis of the Covid-19 pandemic, the GDP referring to the volume of Brazilian agribusiness grew 3.2% in 2020 (CEPEA, 2021). In reference to fish, the State of the World Fisheries and Aquaculture (SOFIA, 2016) estimates that Brazil should register a 104% growth in fishery and aquaculture production by 2025.
Rondônia state is the largest producer of native fishes in Brazil, corresponding to about 47% of the production of native species, with a production in 2020 of 65.5 thousand tons of fishes from fish farming (PEIXE BR, 2021), and has tambaqui (Colossoma macropomum) and pirarucu (Arapaima gigas) as the most cultivated fish which, together, represent about 85% of cultivated fish (MEANTE; DÓRIA, 2017). Pirarucu is a carnivorous fish with excellent growth, which can reach a weight increase of 12.0 kg per year. It is one of the largest freshwater fish in the world, distributed in the Amazon Basin and the northern part of South America (SILVA; DUNCAN, 2016). Pirarucu meat has a smooth flavor, firm texture (ARANTES et al., 2013) and excellent nutritional value, with levels above 6.0 g/100g of lipids, 20g/100g of crude protein and an average of 72% moisture, for the filet mignon (DANTAS FILHO et al., 2021). Its consumption is a traditional habit in the Amazon region, (ONO; KEHDI, 2013), with the salted pirarucu being the most commonly found form of this fish in the regional market, Porto Velho city (SILVA et al., 2016; TORRES; GUEDES, 2017).

The production of shrimp in the Amazon, in turn, unlike fish farming, is at an early stage, requiring support for the development of a production, research and financing system (NUNES et al., 2013). Usually, the shrimp consumed in the Rondônia state come from fishing in rivers, the genus Macrobrachium being the most common, with great variability in morphology and behavior. They are frequently found in the Amazon basin, mainly in floodplain habitats (periodically flooded environments) in white water systems, being rare or absent in places with clear or black waters (COSTA; SILVA, 2001). In the retail market in the Rondônia state, shrimp are usually marketed in salted and/or dry form (VIANA et al., 2016; COZER et al., 2019).

Due to the presence of microorganisms in fish, conservation techniques are imperative. Resources such as heat, salting and drying provide not only an increase in shelf life, but also the generation of products with a new taste and texture (FREITAS et al., 2011). These techniques, when used by the food industry, are performed in a controlled manner, following protocols, generating standardized and safe products. However, artisanal processing prevails among rural populations, who establish their family-run industries (SILVA et al., 2020). In this case, the conditions of hygiene, temperature control and handling can be much less sophisticated (MÁRISICO et al., 2009).

In the salting technique, the method is used in which the muscle is subjected to strong osmotic pressure that begins with the penetration of salt in the superficial part and, little by little, passes to the inner layers, at the same time that the water leaves (FREITAS et al., 2011). Muscle proteins eventually denature and cells contract as a result of dehydration. The reduction in water activity (Aw) of meat ends up inhibiting autolytic and microbial degradations, because it is one of the predominant intrinsic factors that contribute to these degradation processes (BALTAZAR et al., 2013; SILVA et al., 2016).

Given the problem presented, the aimed of the study was to evaluate the hygienic-sanitary characteristics (molds, yeasts and halophilic bacteria) and the moisture content of pirarucu and salted and dry shrimp marketed in retail markets and public fairs in the Porto Velho city, RO - Brazil.

MATERIAL AND METHODS

Bioethical considerations

As the study was carried out with animals slaughtered by those responsible for retail markets and public fairs, it was not necessary to submit the research to the Committee on Ethics in the Use of Animals (CEUA). As it is a product of animal origin, ethical principles in research were respected.

Study area and obtaining samples

The samples were obtained in the Porto Velho city, RO - Brazil, namely, in the Central Market, in two public fairs and in a fish distributor. In the case of the Central Market, due to the proximity of the Madeira River, fish sales are intense. One of the public fairs, Fair A, offers a wide variety of regional products, having been created to serve rural producers. The fish distributor sells fish and shrimp, supplying various fish selling points in the Porto Velho city. The other fair, Fair B, receives most of the fish caught in the region.

A total of 20 fish samples, 10 pirarucu samples and 10 salted and dried shrimp samples, obtained in February and June 2019, were analyzed, and two samples were obtained from each supplier, one in each sample collect. The weights of pirarucu and shrimp samples ranged from 200.0 to 250.0g. At the Central Market and at fairs, the products were on sale at room temperature, corresponding to 25.5°C (02/10/2019) and 23.1°C (06/09/2019) during sample acquisition times. Only in the fish distributor did the products remain refrigerated at 10°C. The samples were transported in the original packaging offered by the merchants, placed in an isothermal container and immediately sent to the Laboratório de Análises Físico-Químicas e Microbiológicas (LAFQM), Universidade Federal de Rondônia (UNIR), em Presidente Médici, RO – Brazil.

Microbiological analysis

Preparation of the research site and samples

In the Laboratory, the work area was disinfected with 70% ethanol to remove any contaminants present. The utensils and instruments used were previously sterilized in an autoclave, sterilization oven or flamed at the time of use. For the analysis
of pirarucu and shrimp samples, 25.0g were weighed, aseptically removed from various points of the acquired pieces.

**Counting of molds and yeasts and halophilic bacteria**

To perform the microbiological analyses, serial dilutions were first prepared according to Silva et al. (2007). For the analysis of molds and yeasts, we used the potato glucose agar culture medium acidified with 10% tartaric acid (SILVA et al., 2007), platting on the surface. This method is based on verifying the capacity of these microorganisms to develop in culture media with a pH close to 3.5 and an incubation temperature of 25°C. The use of acidified media selectively promotes the growth of fungi, inhibiting most bacteria present in the food. Incubation took place at 25.0 ± 1.0°C, for 5 to 7 days, and after this time the colonies were counted.

For halophilic bacteria, Plate Count Agar (PCA) was used with 10% NaCl and surface plating. Incubation was carried out at 25°C for 5 days, according to the compendium of the American Pharmaceutical Association (APHA), described by Baross (2001).

**Method for determining and calculating moisture**

The gravimetric method was used with the use of an oven at 105°C, according to the methodology of the Instituto Adolfo Lutz (IAL, 2008), the method was performed in duplicate.

**Statistical analysis**

Descriptive statistical analysis (arithmetic average and standard deviation) of the microbiological parameters and moisture studied was used. The results were compared with current or recommended legal values for salted and dry fish. To perform the statistical calculations, the program Origin Pro 8.5. This program was linked to the Genes software, version 13.3 (CRUZ, 2013), in which the database was organized.

**RESULTS AND DISCUSSION**

**Molds and yeasts**

The Agência Nacional de Vigilância Sanitária (ANVISA) does not establish parameters for counting molds and yeasts in fish. Therefore, the limit established by the Código Sanitário do Estado de São Paulo (SÃO PAULO, 1978) of 2.69 log UFC/g for salted and dry fish was used. The results for molds and yeasts on pirarucu and shrimp are presented in Table 1.

For pirarucu deboned, the results presented an average of 1.09 log CFU/g, ranging <10 log CFU/g (not detected) to 3 log CFU/g (Table 1 and Figure 1). The samples from the first collections of the Central Market, Feira A and distributor and suppliers expressed values above the limit used as a reference in this study. These contents may indicate that these batches were handled without the use of Good Handling Practices; that there were hygienic-sanitary failures during storage and marketing, with the aggravation of sales being made at room temperature, with the exception of the distributor, which kept them at 10°C. These factors influence the safety of the final product.

According to the Technical Regulation that fixes the identity and quality characteristics of salted and dried fish, in Article 13º it is stated that dried salted fish must be stored and transported at a maximum temperature of 7º C (BRASIL, 2019). This precaution with temperature was not followed at any point of sale, Central market, fairs our distributors. Balthazar et al. (2013) commented that to expose salted fish for sale at room temperature, it would be necessary to store the product in an environment with humidity control. Furthermore, the microbiological quality of the raw material, such as the fish itself, can also interfere, which would explain the higher levels found in the first collection, since there is an interval of 4 months between them, being from different batches. It is worth remembering that the suppliers of these products in the trade in question did not wear a uniform or any garments. Thus, although the salting process is a conservation method, the salted and/or dry product can deteriorate due to molds, which cause “empoado negro”, introduced with the salt itself or by contamination with equipment or utensils sanitized (CALIXTO et al., 2016).

The moisture contents found here were below the results obtained by Vasconcelos et al. (2014), as they obtained an average of 3.5 log CFU/g for molds and yeasts in cuts of dried...
salted pirarucu from open markets in the Manaus city, AM. The authors, although they have not reported the conditions of the pirarucu acquisition sites, point out that artisanal products generated under inadequate conditions, stored and transported in an improper manner, will have compromised microbiological quality. The development of fungi in salted fish can be hazardous to health due to the production of mycotoxins (TOLOSA et al., 2014; MATEJOVA et al., 2016).

In the research carried out by Nunes et al. (2013), fungal growth was detected in dry salted pirarucu deboned from retail markets in the Belém city, PA, with values ranging from 2.60 to 7.08 log CFU/g. Sampels (2015), studying the microbiology of dry salt cod (*Gadus virens*) in the Lisbon city, Portugal, obtained values of up to 6.61 log CFU/g for molds and yeasts, and similar results were found by Gram (2009) in the same region of the Portugal. Sampels (2015) reinforced the need for processing and preservation techniques to be applied in order to contribute to the microbiological and nutritional quality of the product.

Cunha (2018) evaluated molds in salted fish from Luanda city, Angola, in samples of croaker (*Argyrosomus regius*) and horse mackerel (*Tachurus tachurus*) the values were below 2 log UFC/g. In tilapia (*Oreochromis sp*) and catfish (*Pangasiodon gigas*) samples the average was 2.60 log CFU/g. In the yeast counts, mackerel and tilapia species presented <10 log CFU/g, in croaker it ranged 1.23 to 2.37 log CFU/g and in catfish 1.79 to 2.88 log CFU/g. Patir et al. (2006) mentioned the poor hygiene conditions in the production of salt mullet (*Mugil cephalus*) in northern Turkey, in the Lake Van region. The values obtained for molds and yeasts reached 3.24 log CFU/g, compatible with those obtained in the pirarucu analyzed here.

The salted shrimp were for sale under the same temperature and hygiene conditions as the pirarucu. The values for molds and yeasts in samples from all suppliers expressed growth in at least one batch. The values ranged <10 log UFC/g to 3.57 log UFC/g, and the limit of the legislation was exceeded in three samples: from the Central market (1* collect, box x), fair A (1* collect) and distributor (1* collect). Values in the limit of 2.69 log UFC/g were found in the first collect of the Central market supplier (box y) and in the second collect of the distributor and fair B (Table 1 and Figure 2). In the study carried out by Nunes et al. (2013), the authors draw attention to the terrible hygienic and sanitary conditions in the dry salted fish trade in the North region. In the Avuí dried shrimp analyzed by them, coming from supermarkets and open markets in the Belém city, PA, the values reached 7.08 log UFC/g.

Evangelista-Barreto et al. (2016) analyzed shrimp from open markets and the municipal market in the Cruz das Almas city, BA. The fungus load on average was 2.71 log CFU/g. The authors also found pathogens in the product, which makes it a risky food for the community that will consume it without further heat treatment. Among the inadequacy findings were the sale of shrimp in raffia bags and direct exposure to the precarious hygienic-sanitary conditions of the environment (moisture, temperature, dust, insects, birds and domestic animals).

### Halophilic bacteria

Table 2 and Figure 3 present results for the salted and dried pirarucu samples. There are no established limits in national legislation for halophilic bacteria. Some authors consider...
that products with counts above 5 log CFU/g are considered highly contaminated (SILVA et al. 1991). Halophilic bacteria ranged <10 log CFU/g to 6.30 log CFU/g. The only sample that did not have this group of microorganisms was from the second collect at fair B. Values above 5 log CFU/g were found in only two samples.

Halophilic bacteria multiply easily in salty products (NUNES et al., 2012) causing the “cod red” due to the production of the pigment bactorrubein. As mentioned earlier, the products analyzed here were exposed without any protection to environmental conditions. As the microorganisms of the Halobacteriaceae family are mesophilic, room temperature favors their multiplication. Finally, these bacteria produce slime and produce compounds with unpleasant odors in salty food.

Cunha (2018), in his research carried out with salted fish in the Luanda city, Angola, found that there were precarious hygiene practices on the part of handlers, as well as incomplete clothing, with the aggravation of washing their hands infrequently. The author found the following mean results for halophilic in fish: croaker 2.15 log CFU/g, mackerel 2 log CFU/g, tilapia 3.16 log CFU/g and catfish with 2.29 log CFU/g.

Although the need for temperature control for salty fish products is emphasized, Baltazar et al. (2012) when analyzing cod marketed at room temperature and refrigerated in the São Paulo city, SP, found no significant variation in the content of halophilic bacteria in relation to the temperature of storage and sale of the product for 14 days. The values obtained were <10 to 5.38 log CFU/g. A possible explanation, according to the authors, for this non-interference of temperature is related to water activity (Aw), whose values may have prevented microbial multiplication, even at higher temperatures. Thus, although Aw has not been analyzed here, one can think of the possibility that samples from the second batch have lower water activity than those from the first, since these products are handcrafted, that is, they do not undergo a control of the conditions production, which can generate variations in some parameters.
Oliveira et al. (2016), carried out a similar survey in Portugal, evaluated the stability and general appearance of dry salted cod steak packed in air-filled packages and stored at four different temperatures (4, 24, 27 and 30°C). However, the storage time was longer, four weeks. After this period, they observed the development of a red color in the products stored at the two highest temperatures, indicating the development of halophilic bacteria. Issues related to the lack of Good Manufacturing and Sales Practices were addressed by Yam et al. (2015) evaluated halophilic bacteria from various salted and dried fish at markets in Caspian San region, Iran, and detected values of 3 to 4 log CFU/g.

Table 2 and Figure 4 presents the results for halophilic bacteria in shrimp, whose contents ranged <10 to 6.97 log CFU/g. Only in the last collect from the distributor was there no presence of halophilic contaminants in the sample. Figure 4 expresses that in all cases where there was a positive result, the values were high, and in eight collect values above 5 log CFU/g were obtained. In the national and international literature, studies referring to halophilic bacteria in freshwater shrimp were not found, so that comparisons and discussions could be carried out.

Moisture

Normative Instruction No. 1 of January 15, 2019 regulates the percentage of moisture in dry and salted fish, which must be a maximum of 52.9% (BRASIL, 2019). Increased moisture
in the product can occur due to several factors, from handling fish during salting to poor storage conditions for sale.

As mentioned by Baltazar et al. (2013), more than moisture, Aw is an important intrinsic factor regarding the microbiological stability of food products. In the research of these authors, the values of Aw were shown to be stable against environmental variations in temperature and air humidity. Therefore, the authors suggest that Aw should be a parameter of identity and quality of dry fish, rather than moisture.

The results of moisture in the pirarucu cuts (Table 3 and Figure 5) expressed an overall average of 43.38%. Only one sample exceeded the maximum moisture limit and came from the distributor, the first collect, with 54.31%. It should be noted that in the distributor, the fish were kept under refrigeration at 10°C, this temperature being higher than that allowed by Normative Instruction No. 1/2019. The lowest value was from fair B, in the first collect, with 29.94% moisture.

In research carried out by Reis (2015) in the Amazonas state, dry salted pirarucu loin were found to have an average moisture content of 46.52%. The author evaluated the effect of different drying temperatures on the final moisture values. However, in handcrafted products like the ones in this study, this control does not exist, which can lead to a lack of uniformity in the final moisture values, even if these are within legal limits. Marsico et al. (2009) warn about the storage of salted fish and its exposure in places of market, as the relative air humidity of the environment interferes with the absorption of water by the product. The authors analyzed salted and dry cod from retail establishments in the Rio de Janeiro city, RJ, and found an average moisture content of 47.21%.

Aiura et al. (2008) compared the moisture of salted Nile tilapia fillets obtained by dry and wet salting, in the São Paulo city, SP. The results for wet salting were on average 69% and for dry salting 50% on average. Similar values were found by Freitas et al (2011) in pacu fillets (*Piaractus mesopotamicus*), from Santa Helena city, PR, with results of 58.79% for wet salting and 50.75% for dry salting. Therefore, wet salting provides higher moisture values, so that they can exceed the permitted values. It is noteworthy that for shrimp, the FAO recommendations are followed (BELLO; LIMA, 1996), which advises that the moisture should not be higher than 40% for fish in general.

The results found for moisture in the shrimp samples are presented (Table 3 and Figure 6), with an average of 49.22%.

| Supplier/ sample collect | Moisture (%) | Moisture (%) |
|--------------------------|--------------|--------------|
| Central market – box x   |              |              |
| 1st collect              | 37.59 ± 2.63 | 53.83 ± 2.00 |
| 2nd collect              | 38.20 ± 3.32 | 48.76 ± 3.57 |
| Central market – box y   |              |              |
| 1st collect              | 40.15 ± 2.92 | 52.19 ± 0.93 |
| 2nd collect              | 47.12 ± 5.57 | 46.52 ± 2.33 |
| Fair A                   |              |              |
| 1st collect              | 50.92 ± 3.13 | 41.24 ± 0.62 |
| 2nd collect              | 36.99 ± 2.75 | 56.82 ± 1.64 |
| Distributor              |              |              |
| 1st collect              | 54.31 ± 3.95 | 40.6 ± 1.92  |
| 2nd collect              | 51.76 ± 1.52 | 50.85 ± 3.01 |
| Fair B                   |              |              |
| 1st collect              | 29.94 ± 3.35 | 51.67 ± 1.90 |
| 2nd collect              | 44.99 ± 1.55 | 50.64 ± 2.57 |

Caption: green line marks the recommended limit.

Figure 5. Graphs of average moisture values in salted and dried pirarucu marketed in the Porto Velho city, RO - Brazil.
The results ranged between 40.6 and 56.82%, that is, all values above those recommended by the FAO. Kraemer et al (2001) found values between 44.4 and 55.5% in shrimp from retail stores in the Rio de Janeiro state. These high moisture values indicate that the samples were possibly not handled properly during the salting drying processes, or were stored incorrectly, according to the study authors.

Feitosa et al (2018) performed the moisture determination of freshwater shrimp (*Acetes marinus*), dry salty commercialized in open markets in the Santarém city, PA. The results varied between 53.92 and 80.25%, being much higher than recommended by the FAO. Probably, the results were due to the artisanal salting process that can lead to failures in the standardization of the product. Nunes et al (2013) also analyzed dry salted shrimp in a study carried out in the Pará state, finding an average of 24% moisture.

In general, the salting of fish is basically carried out by hand, consisting simply of commercial cuts in the form of fish deboned added at salt and, in most cases, drying in the sun. This process does not standardize the ideal amount of salt, leaving the commercial cuts uneven in terms of salt and moisture.

**CONCLUSIONS**

Microorganism values above the recommended limits may be related to poor conditions in fish processing; the lack of hygiene of utensils and places for manipulation; the poor quality of the raw material used, in addition to exposure to sale without protection in relation to the environment. For moisture, the lack of control over the drying conditions must cause variable values and outside the established standard. However, training in Good Manufacturing Practices, minimum standardization of artisanal salting and drying techniques and the use of quality raw materials can minimize problems in the physicochemical and microbiological quality of fish products from fisheries and aquaculture.

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## REFERENCES

AIURA, F. S. et al. Conservação de filés de tilápia-do-Nilo (*Oreochromis niloticus*) em salga seca e salga úmida. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, v.60, n.6, 2008. doi: 10.1590/S0102-09352008000600033

BALJAZAR, C. et al. Quality of dry salted cod sold at room temperature and refrigerated. *Brazilian Journal of Food Technology*, v.16, p.236-242, 2013. doi: 10.1590/S1981-67232013005000029

BAROSS, J. A. *Compendium of Methods for the Microbiological Examination of Foods*. 4.ed. Washington: American Public Health Association, 2001. 676 p.

BELLO, R. A.; LIMA, C. A. S. Trabajos presentados en la Tercera Consulta de Expertos sobre Tecnología de Productos Pesqueros en la America Latina. Porlamar, Venezuela, 1994. FAO Informe de Pesca nº 538, Supl. Roma: FAO, 1996. p.29-33.
SILVA, N. M. N. et al. Diagnóstico do comércio de pirarucu nos mercados e feiras de Santarém, Pará. *Biota Amazônia*, v.6, n.4, 2016. doi: 10.18561/2179-5746/biotaamazonia.v6n4p49-53

TOLOSA, J. et al. Natural Occurrence of Emerging Fusarium Mycotoxins in Feed and Fish from Aquaculture. *Journal of Agricultural and Food Chemistry*, v.62, n.51, p.12462–12470, 2014. doi: 10.1021/jf5036838

TORRES, G. F.; GUEDES, L. F. O mapeamento de uma cadeia extrativista na Amazônia: Arapaima gigas em reservas de desenvolvimento sustentável. *Revista de Gestão Social e Ambiental*, v.11, n.2, 2017. doi: 10.24857/rgsa.v11i2.1222

VASCONCELOS, E. L. Q. et al. Avaliação microbiológica do pirarucu (Arapaima gigas) salgado seco comercializado em feiras da Cidade de Manaus e Fonte Boa, Amazonas. *Publicações em Medicina Veterinária*, v.8, n.4, 2014.

VIANA, I. C. L. A. et al. Microbiological analysis of tambaqui (*Colossomamacropomum*) marketed in municipal open market of Ariquemes, Rondônia State, Brazil. *Revista Pan-Amazonica de Saúde*, v.7, n.2, p.67-73, 2016. doi: 10.5123/S2176-62232016000200008

YAM, B. A. Z. et al. Microbial quality of salted dried fish sold near Caspian Sea, Iran. *Scholarly Journal of Agricultural Science*, v.5, n.6, p.215-219, 2015.