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Mechanically separated meat prepared with edible tilapia filleting waste from a public fish market

Rafael Gomes Abreu Bacelar*, Nathálya de Oliveira Moura, Marília da Silva Sousa, José Humberto Santos Filho, Karina dos Santos Rodrigues, Eveny Silva de Melo and Maria Christina Sanches Muratori[1]

[1]Departamento de Morfofisiologia Veterinária, Universidade Federal do Piauí, Campus Universitário Ministro Petrônio Portella, Bairro Ininga, 64049-550, Teresina, Piauí, Brazil. *Author for correspondence. E-mail: rafael.bacelar@hotmail.com

ABSTRACT. This study aimed to produce mechanically separated meat (MSM) from tilapia obtained at the Teresina fish market in the state of Piauí, Brazil. Waste production and disposal and yields were estimated, and physico-chemical and microbiological MSM qualities were determined. A questionnaire was applied to the fish market sellers, followed by tilapia carcass sampling for MSM production and assessments concerning yields, microbiological (*Escherichia coli* and *Staphylococcus* coagulase positive counts and the detection of *Salmonella* spp.) and physicochemical (water activity, pH, reaction to hydrogen sulfide and ammonia) quality and proximate composition (moisture, ash, proteins, lipids, carbohydrates and total energy value). The questionnaire results indicated that tilapia is highly in demand and thats processing leads to significant waste. The yield analysis indicated that tilapia MSM presents adequate residual meat extraction rates from tilapia carcasses. All microbiological analyses were in compliance with Brazilian standards. Physicochemical results were as follows: protein (14.9 to 16.1%), lipids (16.6 to 22.1%), ash (1.3 to 2.8%), moisture (60.6 to 64 .1%), carbohydrates (0.6 to 1.5%) and calories (216.5 to 263.1 kcal 100^{-1}). It is, therefore, clear that MSM constitutes a safe and nutritious foodstudd and comprises a viable alternative for the development of co-products.

Keywords: fish pulp; *Oreochromis niloticus*; fish; municipal market.

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Introduction

The Nile Tilapia (*Oreochromis niloticus*) is one of the most cultivated fish species worldwide. A total of 6.2 million tons were produced in 2020, a 2.0% increase compared to the previous year. Brazilian production reached up to 486,155 tons in the same year, a 12.5% growth compared to 2019 (Peixe BR, 2021).

Tilapia are sold either whole, gutted, sliced or filleted. The main tilapia processing method is filleting, performed by removing bony portions, leaving only muscle. Filleting residues can represent 60% of the total fish weight following entrail, head, skin and carcass disposal (Chalamaiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012). Tilapia carcasses contain edible residues in the form of muscle attached to the backbone (ventral abdominal muscle and deep hypoaxial muscle) and spine trimmings (Gjerde, Mengistu, Ødegård, Johansen, & Altamirano, 2012; Oliveira Filho, Viegas, Kamimura, & Trindade, 2012).

The use of edible filleting residues avoids waste while also reducing production costs and environmental pollution. The development of technological alternatives regarding fish waste allows for diferente food preparations, als favoring new employment opportunities and sustainable development (Bernadino Filho & Xavier, 2019). In addition, tilapia carcasses present high nutritional value, containing significant protein and lipid contents (Bucker et al., 2020).

Mechanically separated meat (MSM) is obtained from fish bone trimmings through processing in pulping equipment, obtaining muscle particles free of bones, entrails, scales and skin, resulting in a higher yields than simple filleting (Bernardino Filho & Xavier, 2019).

Public markets that sell *in natura* fish are considered important waste generators, mainly due to fish filleting. This type of processing is linked to customer demands when purchasing the fish, to not to take this waste home. This leads to the accumulation of high amounts of waste discarded in these markets, reaching up to about one and a half tons every day, according to local work routines (Costa & Souza, 2012; Souza, Silva, Canto, & Pontes, 2019).

In view of the above, this study aimed to estimate waste production and disposal in the Teresina fish market, in the state of Piauí, Brazil, applying a questionnaire and preparing MSM from discarded tilapia carcasses after filleting by market workers. MSM yields and physico-chemical and microbiological quality assessments were also carried out.

Material and methods

A first visit was made to the Teresina fish market in the state of Piauí, Brazil (05°05′21″S; 42°48′05″W), which functions as a warehouse that sells and distributes several fish species.

Questionnaire

Data were obtained qualitatively by applying a previously prepared questionnaire to local market traders to identify the main marketed fish species, fish processing techniques and filleting residues, including storage, marketing, potential losses and final destination. The questionnaire comprised seven open questions designed and tested before being effectively applied.

After applying the questionnaire to 14 market stall merchants, the stall presenting the highest daily sales was chosen for the fish sampling. Ten Nile tilapia carcasses resulting from manual filleting performed by stall sellers were acquired. The carcasses were beheaded and skinless and contained muscle tissue adhered to bony portions (backbone, ribs and fin bones). Sampling took place fortnightly on seven Saturdays, from March to May 2019, when the highest sales were observed, totaling seventy carcasses. The carcasses were stored in an isothermal box containing recyclable ice ande transpordt to the Fish Technology Sector belonging to the Center for Studies and Research and Food Processing (NUEPPA), at the Federal University of Piauí (UFPI).

This project was approved by the UFPI Human Research Ethics Committee (CEP), Certificate of Presentation for Ethical Assessment (CAAE) no. 89732318.3.0000.5214, concerning questionnaire application to the Teresina fish market traders, Piauí, Brazil.

MSM preparation

Tilapia MSM was obtained using a compact SPx100 fish pulper developed by the UFPI NUEPPA and Production Engineering team, manufactured by a local steel mill (Figure 1). This small machine was especially designed for the development of this project, to be used for MSM preparation by market traders and small filleting companies that generate edible fish waste.

Source: author's collection.

Figure 1. Pulping machine developed to obtain MSM from discarded tilapia carcasses after filleting in a public market in Teresina, Piauí.

Yield analysis

Any entrail remains of the sampled tilapia carcasses were removed, followed by washing under running water and immersion in a hyperchlorinated solution containing 5.0 ppm free residual chlorine for ten minutes. The carcasses were then weighed and processed in the aforementioned fish pulper and the obtained MSM was weighed for yield calculations, according to the following formula:

$$\text{Yield (\%)} = \frac{\text{MSM weight}}{\text{Carcass weight}} \text{ x } 100$$

Five 200 g portions were aseptically removed from each MSM sample for microbiological analyses and another five for physicochemical analyses. The MSM production flowchart applied herein is displayed in Figure 2.

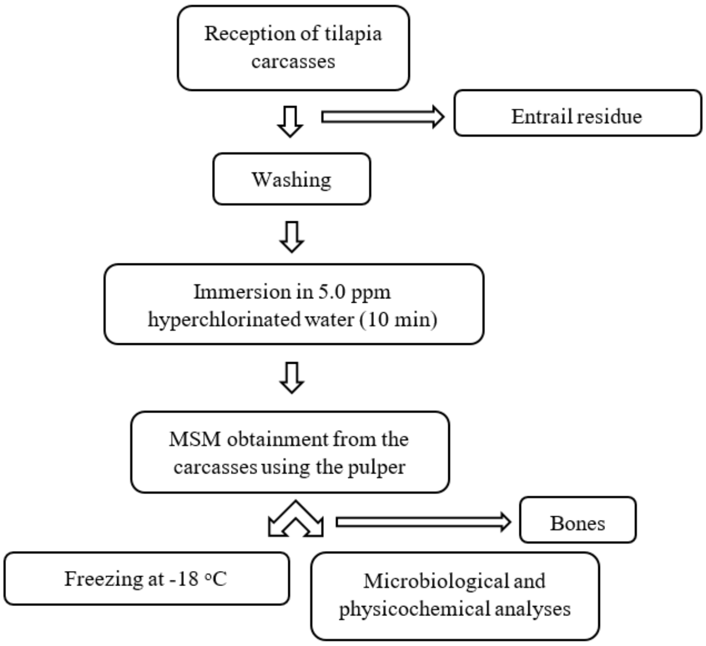

Figure 2. Flowchart concerning MSM production from discarded tilapia carcasses after filleting at the Teresina fish market, Piauí, Brazil.

Microbiological analyses

All microbiological analyses were performed following American Public Health Association (APHA, 2015) standards for coagulase positive *Staphylococcus*, *Salmonella* spp. and *Escherichia coli* according to Brazilian RDC nº 60 (Brasil, 2019).

Physico-chemical analyses

All physicochemical analyses were performed in triplicate. The micro-Kjeldahl method was used determine protein content, applying a nitrogen protein factor of 6.25 (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2014a). Lipid content determinations were performed using a Sohxlet extractor employing petroleum ether (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2014b). Moisture contents were determined by drying at 105°C in an oven until obtaining constant sample weight, and ash contents were determined by incineration in a muffle furnace at 550°C for four hours (Instituto Adolfo Lutz, 2008). Carbohydrate contents were determined by adding up moisture, protein, lipid and ash values and subtracting them by 100. Calorie determinations were performed by applying the following conversion factors: 4 kcal g^{-1} for protein, 4 kcal g^{-1} for carbohydrates and 9 kcal g^{-1} for lipids (Brasil, 2020). The water activity analysis was performed using a portable digital Decagon Pawkit device, using 5.0 g of each sample.

For the pH analysis, 10 g of each sample were transferred to a beaker and mixed with 100 mL of distilled water and pH readings were taken using a Hanna 21 pH/mV meter by introducing the device electrode into each sample.

Ammonia determinations were performed by observing the presence or absence of white vapor in test tubes formed by reactions with ammonium chloride (Instituto Adolfo Lutz, 2008). The Eber test for hydrogen sulfide (H_2S) was performed by comparing the sample stain with a sodium sulfide monohydrate ($Na_2S.9H_2O$) standard (Instituto Adolfo Lutz, 2008).

Statistical analyses

Data normality was first verified, followed by an analysis of variance (ANOVA), applying the Holm-Sidak test to compare potentially significant differences between the means of each variable, adopting a significance level of 5.0%. Microbiological data were normalized to $log^{10(x+1)}$. All statistical analyses were performed using the Sigmastat 4.0 software.

Results and discussion

Questionnaire

The most commercialized fish species at the Teresina fish market according to the answered questionnaires are presented in Figure 3.

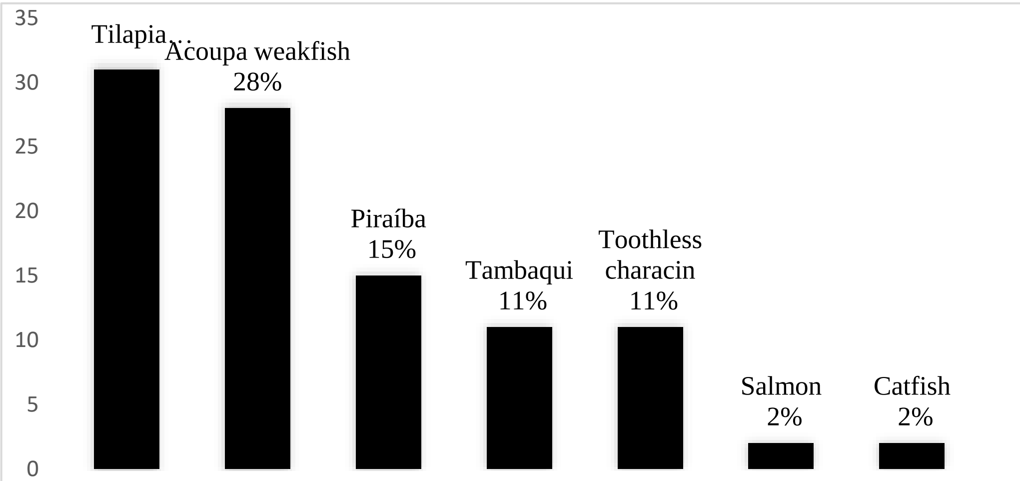

Figure 3. The most commercialized fish species at the Teresina fish market, PI according the the answered questionnaires.

Interviewed merchants reported that the most sold fish species at the fish market are Tilapia (*Oreochromis* sp., 31%), Acoupa weakfish (*Cynoscion acoupa,* 28%), Piraíba (*Brachyplathystoma filamentosum,* 15%), Tambaqui (*Colossoma macropomum,* 11%), toothless characin (*Curimatidae,* 11%), Salmon (*Salmo salar* L., 2%) and catfish (*Pseudoplatystoma* sp., 2%).

Consumers required the following fish cleaning processes: peeling (42.1%), filleting (31.6%) and evisceration (26.3%), all of which generate waste. The market has a specific area for organic waste storage, aiming to reduce the presence of vultures, cats, dogs, rats, and insects, among others.

Merchants also indicated that the edible fish waste generated by filleting in their stalls is destined to various causes, such as donations (41.4%), sales (34.5%), their own consumption (13.8%), customer delivery (6.9%) and disposal (3.4%). In this regard, Souza et al. (2019) reported that filleting was the most requested processing by local consumers in a fish market in Belém, in the state of Pará, Brazil, and that the remaining carcasses were either donated or discarded in a sanitary landfill, similar to the present study.

The interviews also aimed to obtain information on how the edible fish waste was stored prior to its final disposal. Most merchants reported freezing the waste (-18°C) in domestic freezers in their stalls (64.3%), while some stored the waste in isothermal boxes containing ice (28.6%) or exposed at room temperature in containers (7.1%).

Regarding the destination of inedible fish waste (entrails, bones, scales andg skin), the interviewees indicate storing it in their own garbage containers until taking it to the disposal area outside the market. According to these respondents, the final destination to the sanitary landfill is carried out by the municipal government through the public collection system. Non-edible waste also comprises raw material that could be used to prepare by-products such as compost, silage and flour, which would, in turn, reduce environmental problems and demands for opening new landfill areas, also decreasing transportation costs.

Yield analysis

The tilapia MSM yield results obtained from discarded tilapia carcasses after filleting by Teresina fish market merchants are displayed in Table 1.

Table 1. MSM yields from discarded tilapia carcasses after filleting by Teresina fish market sellers in Teresina, Piauí, Brazil.

Collect	Carcasse (g)	CV	MSM (g)	CV	Yield (%)
1	$229,6^{a}\pm35,2$	15,3	$125,4^{a}\pm27,0$	21,5	$54,4^{a}\pm7,4$
2	$167,5^{b}\pm20,2$	12,1	$78,2^{b}\pm20,0$	25,6	$46,2^{a}\pm8,1$
3	$192,8^{ab}\pm28,4$	14,7	$95,1^{b}\pm19,9$	20,9	$46,2^{a}\pm8,6$
4	$211,9^{a}\pm30,5$	14,4	$95,7^{b}\pm14,8$	15,5	$45,5^{a}\pm6,7$
5	$203,5^{ab}\pm18,8$	9,2	$99,7^{b}\pm11,5$	11,5	$49,1^{a}\pm5,0$
6	$196,1^{ab}\pm20,9$	10,7	$93,0^{b}\pm13,1$	14,1	$47,3^{a}\pm4,0$
7	$196,1^{ab}\pm33,5$	17,1	$87,0^{b}\pm18,5$	21,3	$47,3^{a}\pm5,0$
Total	14,3 kg		6,8 kg		

Means on the same line followed by different letters differ from each other by the Holm-Sidak test ($p < 0.05$). Data are expressed as means ± standard deviation. CV = Coefficient of variation.

Significant differences were observed when comparing sampled carcasses ($p < 0.05$), due to different sampling dates and suppliers and individual fish variations according to the harvesting period, as animals exhibit variable weights during marketing. In this regard, Kirschnik and Macedo-Viegas (2009) highlight that MSM production also represents a viable way to obtain the most of the production potential of fish that have not yet reached the ideal average weight for commercialization.

The use of fish carcasses varies according to species, shape, size and processing method (Rossato et al., 2018). The MSM yields of tilapia carcasses waweres not significantly different ($p < 0.05$), even though samples were obtained on different days. Average yields ranged between 46.2 and 54.4% (Table 1), close to tilapia carcass MSM yields reported by Kirschnik and Macedo-Viegas (2009) and Gryschek, Oetterer, and Gallo (2003), of 46.9 and 51.7%, respectively.

The amount of extracted meat and MSM quality depend on carcass cleanliness, the type of pulper and the size of the pulper cylinder holes. Adequate MSM yields and decreased levels of connective tissue, skin, bone and scale are obtained using pulps with cylinder holes between 1.0 and 2.0 mm, as reduced muscle particle sizes favor muscle disintegration, altering final MSM color and texture (Tenuta-Filho & Jesus, 2003). The cylinder holes of the pulper employed herein for MSM production measure 1.19 mm, ensuring was uniform MSM consistency and color without the presence of bones and connective tissue.

A total of 14.3 kg of carcasses (seventy specimens) were obtained in the seven days of market research, totaling 6.8 kg of MSM (Table 1). This amount comprised 34.5% of the daily edible waste destined for direct customer sales in the selected fish sale stall. Traders indicating discarding 3.4% of waste a day, which could indicate extra income if this were used to produce and sell MSM.

Microbiological analyses

The microbiological analysis results of the MSM tilapia samples were not significantly different ($p < 0.05$) concerning *Escherichia coli* coagulase positive, *Staphylococcus* count and *Salmonella* spp. counts (Table 2).

Table 2. Microbiological MSM analyses from discarded tilapia carcasses after filleting performed by Teresina fish market sellers in Teresina, Piauí, Brazil.

Collect	Coagulase-positive *Staphylococcus* (CFU g^{-1})	*Escherichia coli* (MPN g^{-1})	*Salmonella* spp.
1	<1,0	<0,3	Absent
2	<1,0	<0,3	Absent
3	0,6±1,5	<0,3	Absent
4	1,4±1,9	<0,3	Absent
5	1,3±1,8	<0,3	Absent
Legislation (Brasil, 2019)	3,0	2,7	Absent /25g

CFU g^{-1} = Colony Forming Units per gram in logarithmic numbers. Probability ($p < 0.05$). MPN g^{-1}: Most Probable Number per gram in logarithmic numbers.

These results are in accordance with the National Health Surveillance Agency standards established by normative instruction no. 60 of December 23, 2019 (Brazil, 2019), indicating that all samples, were, therefore fit for human consumption. Acceptable microbiological results for tilapia MSM were also reported by Lustosa-Neto et al. (2018) and Fogaça, Otani, Portella, Santos-Filho, and Sant'ana (2015). Although the tilapia carcasses were obtained from a public market, all obtained MSM presented adequate microbiological quality (Table 2), probably due to good manufacturing practices during all processing stages (Figure 2), and reduction of pre-existing microbiota due to immersion in hyperchlorinated water, since microbiological MSM conditions are associated to several factors, including raw material quality and good manufacturing practices during sample processing.

Physicochemical analyses

The tilapia MSM proximate composition results are presented in Table 3.

Table 3. Centesimal MSM composition from discarded tilapia carcasses after filleting performed by Teresina fish market sellers in Teresina, Piauí, Brazil.

Collect	Parameters (%)					
	Protein	Lipids	Carbohydrates	Moisture	Ash	Total Energy Value (TEV)
1	$16,1^{a}$±0,2	$16,6^{c}$±1,1	$0,8^{a}$±0,8	$64,1^{a}$±0,8	$2,8^{a}$±0,04	$216,5^{c}$±5,8
2	$15,5^{a}$±0,5	$19,7^{b}$±0,6	$0,6^{a}$±0,5	$61,4^{b}$±1,0	$2,7^{a}$±0,09	$242,3^{b}$±6,7
3	$15,4^{a}$±2,4	$19,9^{b}$±0,6	$1,2^{a}$±1,9	$61,4^{b}$±0,4	$2,3^{b}$±0,02	$246,3^{b}$±4,0
4	$14,9^{a}$±1,3	$19,3^{b}$±0,1	$1,5^{a}$±1,9	$63,4^{ab}$±2,0	$1,6^{b}$±0,21	$238,9^{b}$±8,5
5	$15,1^{a}$±1,04	$22,1^{a}$±0.1	$0,9^{a}$±1,0	$60,6^{b}$±0,7	$1,3^{c}$±0,07	$263,1^{a}$±6,0

Means on the same line followed by different letters differ from each other by the Holm-Sidak test ($p < 0.05$). Data are expressed as means ± standard deviation. *: values expressed in kilocalories (kcal) in 100 g of sample.

The obtained tilapia MSM samples all presented similar protein percentages ($p < 0.05$) (Table 3), within the expected range for tilapia muscle, ranging from 10.75 to 17.74% (Bernadino Filho & Xavier, 2019). Tilapia MSM is a source of myofibrillar, sarcoplasmic and stromatic protein that can serve as a basis for the preparation of diveseveralrse food products such as protein concentrates, surimi, kamaboko, sausages or emulsified and breaded presentations, among others (Kirschnik & Macedo-Viegas, 2009; Gonçalves, 2011; Rebouças et al., 2012).

Lipid MSM levels ranged from 16.6 to 22.1% (Table 3). In general, tilapia muscle tissue can contain from 2.17 to 15.37% lipid content (Bernadino Filho & Xavier, 2019). Physiological aspects, diet and season of the year are determining factors for lipid levels in fish (Bordignon et al., 2010; Vidotti & Martins, 2010). However, the filleting process can leave adipose tissue adhered to the carcass, consequently increasing lipid percentages (Dallabona et al., 2013). Thus, the values obtained in the present study can be explained by remaining adipose tissue in the abdominal muscle tissue of the carcasses used in MSM obtainment.

Fish muscle tissue is characterized by low carbohydrate levels, usually between 0.3 and 1.0%, mainly in the form of glycogen and mucopolysaccharides (Ogawa & Maia, 1999; Minozzo, 2011). Carbohydrates ranged from 0.6 to 1.5% in the present study (Table 3), probably due to residual glycogen in the muscle of freshly slaughtered fish from local fish farmers and the cold chain maintenance. The crushing of muscle fibers during MSM extraction may have also favored carbohydrate maintenance in tilapia muslce.

A significant difference was noted between samplings ($p < 0.05$) regarding MSM moisture content, which ranged between 60.6 and 64.1% (Table 3). Other studies report tilapia MSM moisture values between 59.36% (Macedo, Silva, & Apolinário, 2020) and 76.9% (Scudeler, Costa, Cortez-Veja, Prentice, & Fonseca, 2020). The observed values are, thus, within the expected range, and the applied storage conditions should be maintained, as this parameter directly influences sample shelf life, hygienic-sanitary and organoleptic quality, with conservation techniques, thus, able to guarantee the required stability of the raw material (Oliveira et al., 2013).

FIsh ash content can vary according to species, age, sex, nutritional aspects and type of rearing (Bery, Nunes, Silva, Santos, & Bery, 2012), and can range from 0.1 to 3.3% (Contreras Guzmán, 2002). However, tilapia MSM ash concentrations are also influenced by the pulping process, and may increase depending on the percentage of ground bones during the preparation of this raw material (Daga et al., 2020).

In general, tilapia muscle provides 117 kcal 100 g^{-1} (Ferreira, Militani, & Duarte, 2012). Calories from tilapia MSM were significantly different ($p < 0.05$), ranging from 216.5 to 263.1 kcal 100 g^{-1}, mainly due to the high levels of lipids (Table 3), probably due to the use of ventral abdominal muscles attached to the carcasses in the MSM preparation.

The proximate composition of the employed tilapia carcasses exhibited variations, probably influenced by several factors in addition to the fish constitution itself, including carcass quality and remaining fatty tissue in the abdominal wall, as well as fragmented bones and fin residues. Other factors that may interfere in tilapia proximate composition are associated to processing, such as filleting method, type of pulper used for MSM production, pulp hole-opening and pulp bone-separation capability.

The tilapia MSM pH, water activity and gas (hydrogen sulphide and ammonia) results are displayed in Table 4.

Table 4. Hydrogen sulfide, ammonia, water activity and pH results for tilapia MSM from discarded tilapia carcasses after filleting performed by Teresina fish market sellers in Teresina, Piauí, Brazil.

Collect	pH	Water activity	Hydrogen sulfide reactions	Test for ammonia
1	$6,1^{a}\pm0,06$	$0,81^{a}\pm0,02$	Negative	Negative
2	$6,0^{a}\pm0,07$	$0,82^{a}\pm0,01$	Negative	Negative
3	$6,1^{a}\pm0,06$	$0,82^{a}\pm0,01$	Negative	Negative
4	$6,1^{a}\pm0,05$	$0,83^{a}\pm0,02$	Negative	Negative
5	$6,1^{a}\pm0,05$	$0,83^{a}\pm0,01$	Negative	Negative
Legislation (Brasil, 2017)	<7,0	-	-	-

Tilapia MSM pH values ranged from 6.0 to 6.2 (Table 4), with no significant difference ($p < 0.05$) between sampling days. These values are in accordance with the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin – RIISPOA, which recommends values lower than 7.0 for fresh fish meat (Brasil, 2017). pH values interfere with the ability of microorganisms to multiply in foods, and low acidity (pH > 4.5) values favor the development of pathogenic and spoilage microorganisms (Fiorda & Siqueira 2009). Factors such as fish species, protein decomposition, microbial loads and capture, handling and storage conditions can influence pH variations in fish in general (Muratori, Viana, Rodrigues, & Júnior, 2004). Despite the low acidity of the produced tilapia MSM, the observed pH results indicate that chemical and microbiological freshness conditions are preserved in tilapia fish market carcasses after filleting.

Water activities (Aw) in the MSM samples (Table 4) were not significantly differet ($p < 0.05$) between sampling occasions. High water activity values in foodstuffs favor chemical and biochemical reactions (Melo Filho & Vasconcelos, 2011) and microbial multiplication. The minimum Aw values for microorganism development are set as 0.91 for bacteria in general, 0.88 for yeast, and 0.80 for filamentous fungi (Maia, Belisário, Carvalho, & Cavalcante, 2020). The values between 0.81 and 0.83 observed for the MSM samples in the present study characterize this raw material as susceptible to fungi multiplication, requiring conservation under controlled conditions to maintain intrinsic quality characteristics.

All samples were negative for hydrogen sulfide and ammonia, indicating safe MSM consumption of tilapia carcass MSM. Positive hydrogen sulfide reactions in fish indicate advanced deterioration, reflected by the degradation of sulfur amino acids inherent to microbial development and inadequate conservation (Rodrigues et al., 2012). The Eber test for ammonia is also used to assess fish conservation status, where ammonia release is indicative of the beginning of protein degradation through amino acid deamination and nucleotide degradation (Zenebon, Pascuet, & Tiglea, 2008; Monteiro et al., 2008; Monteiro et al. al., 2012).

Based on the microbiological and freshness indicators adopted herein, the MSM obtained from tilapia carcasses was adequate for human consumption, probably due daily fish supplies to the market. In addition, tilapia are marketed quickly due to local acceptance, favoring good raw material quality. Furthermore, fish sellers keep fish at close to ice temperature during marketing and attempt to comply with good handling practices during tilapia filleting.

Conclusion

The tilapia MSM prepared with edible residues from filleting at the Teresina fish market exhibited adequate microbiological and physico-chemical characteristics, constituting a safe and nutritious foodstuff.

Tilapia MSM is, thus, a viable alternative for the use of residual meat from tilapia ecarcasss and as a raw material for co-product development, contributing to decreased food waste and environmental contamination.

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