

## Development and evaluation of fresh sausage type of marine catfish [*Sciades herzbergii* (Bloch, 1794)] stored under low temperatures

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### Abstract

The objective of the present work was to develop and evaluate the quality aspects of fresh sausage from marine catfish (*Sciades herzbergii*) stored under low temperatures. The formulated sausage yielded 59.6% moisture, 18.9% protein, 11.6% lipid, 3.0% ash, 6.8% carbohydrates, acceptability index between 90.2% to 82.7%, and purchase intent of "probably would buy". During refrigeration storage, there was an increase in lipid oxidation, volatile bases nitrogen, redness ( $a^*$ ), and reduced water-holding capacity. *Staphylococcus* coagulase positive were  $< 2$  log CFU/g and *E. coli* increased. During frozen storage, there was an increase in lipid oxidation and volatile bases nitrogen. The values of  $L^*$ ,  $a^*$  and  $b^*$  decreased. *E. coli* and *Staphylococcus* coagulase positive were  $< 2$  log CFU/g. Therefore, the sausages of marine catfish featured good nutritional quality, sensory, and shelf life between 21 to 25 days when kept under refrigeration and four months under freezing.

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### Introduction

The change in the profile of food consumers is growing. The desire for products that have good cost:benefit ratio as well as nutritional and sensory quality encourages the market to seek for better food alternatives (Allothman *et al.*, 2009). The increase in the per capita consumption of fish from 9.9 kg in the 1960's to 20.1 kg in 2014 (FAO, 2016) ratifies the growing need of consumers. Fish generally has good nutritional quality due to its high amounts of protein and essential amino acids, vitamins, minerals and essential fatty acids. Another important factor is its protein digestibility which ranges from 90 to 95% (Oliveira Filho *et al.*, 2010b). World fisheries production in marine waters was 79.7 million tons in 2012. With the production of 550,000 tons, Brazil represents 0.7% of the world's fisheries extraction (Brasil, 2011). Among the captured fish producing regions, north eastern Brazil was more productive, with about 35% of the national production, with its State of Pernambuco at the 11<sup>th</sup> position in Brazil,

having a production of approximately 10,000 tons, equivalent to 2% of the national amount. Among the types of fish caught by the marine fisheries, 87% are marine catfish, representing approximately 2% of the entire production (Brasil, 2011).

The catfish comprises of more than 2,700 species, subdivided into 34 families, among which the Ariidae inhabits salt water (Diogo, 2004). One catfish species, *Sciades herzbergii*, which is from family Ariidae, is widely distributed in tropical and subtropical waters, including marine environments, estuarine and lacustrine (Machado *et al.*, 2012). Their body is elongated, without the presence of scales, with a single dorsal fin whose first ray is a thorn-aliasing, and barbels that assist in the apprehension of the food. They are characterised as a kind of fishery caught with other species, such as shrimp, with few associated studies when compared with the freshwater catfishes (Denadai *et al.*, 2013). Therefore, ways to add value to marine catfish, as a raw material in the production of ready-to-eat products, are of great interest, in order to offer value-added products and to address sustainability.

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Sausages are processed meat products obtained from butchered animal meat, to which may be added fatty tissues, ingredients, built in natural or artificial wrap, and is then sent to the appropriate technological process (Brasil, 2000). The type of sausage varies according to the manufacturing technology, in the form of fresh, dried, cured or cooked. There are few studies evaluating the stability of sausages from fish meat. Among them, one can mention the *vongole* (*Anomalocardia brasiliiana*) sausages that have been studied from an aspect of sensory acceptance and stability when stored under freezing (-18°C) (Bispo et al., 2004). The sausage presented above 70% in acceptability aspects of texture and flavour, and good physical-chemical and microbiological stability. In another study, the physical-chemical and microbiological aspects have been assessed in commercial sausages prepared with meat of *Lutjanus erythropterus* or prepared with meat of *Argyrosomus heinii* with supplementation of spices and stored for up to 12 weeks at -20°C. The authors observed that the addition of spices decreased the microbial count and lipid oxidation, and retained the best aspects of colour during the proposed storage period (Al Bulushi et al., 2013).

Although there are few studies evaluating the quality and stability of fish sausages, studies are still lacking in evaluating the aspects of quality and stability of embedded type fresh sausage made with meat of marine catfish (*S. herzbergii*). Therefore, the objective of the present work was to develop and evaluate the quality and stability aspects of sausages of type frescal made from marine catfish and stored under low temperatures.

## Materials and methods

### Raw materials

Approximately 33 kg marine catfish of average weight of  $315 \pm 186$  g with maximum freshness were harvested from artisanal fishing in the municipality of Sirinhaém, State of Pernambuco, Brazil (Latitude: 08° 35 ' 27 "S, Longitude: 35° 58 ' 06" W). The fish were packed in coolers with ice flakes and taken to the Laboratory of Fish Technology, belonging to the Department of Fisheries and Aquaculture of the UFRPE, Recife, PE. In the laboratory, the fish were washed with chlorinated water (5 ppm) for removal of the superficial mucus and then filleted (20% yield of fillet). Subsequently, the fillets were frozen in freezer (-20°C) and maintained for 7 d prior to the preparation of sausages.

### Formulations

The formulations were calculated for 3.3 kg catfish sausage for each treatment, differing as to the type of storage (cooled to 6°C or frozen at -20°C). The ingredients used for the preparation of sausages were added according to preliminary trials: marine catfish fillets (82%), bacon (16%), salt (NaCl) (0.3%), fresh sausage seasoning (1%) (Tuscan Condiment, Kraki®), curing salt (0.2%) (Master healing, BRC® Ingredients – salt and sodium nitrite), stabiliser (0.25%) (Master Fos, BRC® Ingredients – sodium tripolyphosphate), and antioxidant (0.25%) (Master Fix – BRC® Ingredients – sucrose, sodium erythorbate and ascorbic acid).

### Processing of sausages

The catfish fillets were defrosted for about 24 h to  $6 \pm 2^\circ\text{C}$ , weighed, and ground into a meat grinder with 6 mm diameter disk. After the grinding was complete, the meat was mixed with other ingredients in a mixer. Porcine inlay with 30-32 mm gauge were used for natural casing and were first unleashed for at least 2 h in advance and tied by hand so that each sausage was approximately 6 cm long. The sausages were then packed in sterile polyethylene bags (Nylon Poli -18 × 25 × 0.12 cm, 120 µm), each containing three units of sausages, submitted to 720 mmHg vacuum of pressure for 25 s, where half was stored frozen at  $-20 \pm 2^\circ\text{C}$  for up to four months (stability analyses carried out at 0, 1, 2, 3 and 4 months) and the other half stored at  $6 \pm 2^\circ\text{C}$  for up to 32 d (stability analyses carried out at 4, 11, 18, 25 and 32 days).

### Chemical-nutritional composition

The chemical-nutritional composition of the sausages was determined in accordance with the official AOAC methodology (2012) at time 0, both for the frozen and refrigeration storage. The crude protein was determined by the Kjeldahl method ( $\text{N} \times 6.25$ ), and the fat determination was performed by a Soxhlet type extractor using petroleum ether as solvent extraction. The moisture was measured using an oven at 105°C with air circulation until constant weight was attained. The ash content was determined by means of incineration in a muffle furnace at 550°C for 5 h. The carbohydrates were calculated by subtracting the moisture, protein, fat and ash percentages from total.

### Sensory evaluation

The sensory evaluation was performed after microbiological analysis in order to maintain the safety of assessors. The analysis was performed in the laboratory equipped with individual cabins with

white fluorescent light. The sausages were previously roasted on an electric BBQ grill until the internal temperature reaches 90°C, measured with the aid of a skewer-type thermometer and kept the 70°C in electric oven. The sausages were sliced every 2 cm along their length, and served two pieces of sausage per panellist. Affective acceptance tests were carried out by 132 untrained panellists, recruited randomly between students, staff and teachers of the UFRPE, using the methodology described by Meilgaard *et al.* (2006). The sensory attributes evaluated were colour, odour, texture, flavour and overall acceptance, using a hedonic scale of 9 points (9- really liked to 1- really disliked). With the results of the analysis of the attributes of colour, odour, texture, flavour and overall acceptance, the acceptability indices (AI) were calculated according to the method of Dutcosky (1996):

$$AI = \frac{\text{average mark obtained for the product}}{\text{maximum score achieved}} \times 100$$

The intention to purchase was also evaluated using a hedonic scale of 5 points (5 - would certainly buy, to 1- would certainly not buy). The study was approved in advance by the ethics on Research Committee of the University of Pernambuco/PROPEGE, certificate No 637,490 (CAAE: 24094213.9.0000.5207), in accordance with resolution 196/96 of the Ministry of Health of Brazil (Brasil, 1996).

#### *Physico-chemical and microbiological stability*

##### *Lipid Oxidation (TBARS)*

The lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method, in triplicate, in accordance to Vyncke (1970). For the calculation of the values of TBARS, a standard curve for tetramethoxypropane was obtained, and the results were expressed as mg malonaldehyde/kg sample.

##### *Total Volatile Bases Nitrogen (TVB-N)*

The TVB-N was determined in triplicate following the Howgate method (1976). The test result was calculated according to the formula:

$$TVB-N \text{ (mg N/100 g)} = \frac{[\text{volume HCL (mL)} \times \text{normality of HCL} \times 14 \times \text{extraction volume TCA} \times 100]}{(25 \times \text{sample weight})}$$

##### *Water Holding Capacity (WHC)*

For the analysis of WHC, 5 g sample were weighed in triplicate, placed on qualitative filter papers ( $\phi$  125

mm), placed in Falcon tubes and centrifuged at 3,500 rpm for 10 min. After centrifugation, the samples were carefully removed onto heavy papers, and the WHC was calculated according to Grau and Hamm (1953) using the following equation:

$$\% \text{ WHC} = \frac{(\text{Sample weight after centrifugation})}{(\text{Sample weight before centrifugation})} \times 100$$

##### *Determination of pH*

The pH was determined with a potentiometer (Tecnal, model Tec-3MP2), in a solution of 10 g sample previously homogenised with 40 mL distilled water, according to the methods of Oliveira Filho *et al.* (2012).

##### *Instrumental colour*

The instrumental colour was determined in the inner part of three sausages of every treatment using a portable colorimeter CR model 400 (Konica Minolta®), previously calibrated with a standard white before each analysis. The colorimeter employed a xenon lamp having an illumination C ( $Y = 92.78$ ;  $x = 0.3139$ ;  $y = 0.3200$ ), angle of view of 40°, and range of 8 mm in diameter. The colour was expressed using the CIELab system colour patterns-"Commission Internationale de L'Eclairage":  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness).

##### *Microbiological analysis*

For microbiological tests, samples of sausages in triplicate for each treatment were collected and weighed aseptically, homogenised, and grown in specific sterile caps, according to the methods of the Normative 62 the Ministry of Agriculture Livestock and Supply-MAPA (Brasil, 2003).

For total aerobic bacteria count, psychrotrophic, thermotolerants coliforms (*Escherichia coli*), Coagulase positive *Staphylococcus* and *Salmonella* spp. Compact Dry® commercial kits were used (Compact Dry TC®, Compact Dry EC®, Compact Dry XSA®, Compact Dry SL®) to rapidly identify microorganisms based on methods approved by Codex Alimentarius, I.C.M.S.F., APHA, FDA, IT Standards and Bacteriological testing standards, and the AOAC for food. The results were later compared according to the requirements of the current legislation (ANVISA, 2001) in Brazil.

##### *Experimental design and statistical analysis*

Analyses were performed separately in the sausages stored under refrigeration (6°C) or freezing (-20°C). The experimental design used for physicochemical analyses was completely

randomised design for both treatments (chilled and frozen) in three replicas (each sample with 50 g of sausage) at each point of analysis (frozen sausages - 0, 1, 2, 3 and 4 months and refrigerated sausages - 4, 11, 18, 25 and 32 days). The results were initially analysed for normality using the Shapiro-Wilk test, and scapular of the variability with the Bartlett test. After this analysis, analysis of variance (ANOVA) was performed using a 5% level of significance with trend studies of linear or polynomial regressions (physical-chemical analysis), based on the choice of the best coefficient of determination ( $R^2$  adjusted), and Tukey's test for bacteriological analysis. The statistical analyses were performed with the program R (R Core Team, 2017), which is an open source and freely available.

## Results and discussion

### Chemical-nutritional composition

The flesh of fish typically has between 60 to 85% humidity (Contreras-Guzmán, 2002). The sausages of marine catfish analysed in the present work yielded  $59.59 \pm 0.53\%$  of moisture (Table 1). The borderline low moisture level might have occurred due to the addition of bacon in the formulation, which is rich in fat and its function improves the juiciness and flavour. Sausages prepared with mechanically separated fish meat (MSM) obtained from Nile tilapia (*Oreochromis niloticus*) filleting waste had 54.26 to 61.18% humidity (Dallabona et al., 2013), i.e., close to that observed in the present work. The smallest percentage of moisture of the meat products in relation to meat in *natura* can provide a greater product life time, due to reduced water activity which diminishes the possibility of microbial development. The Brazilian legislation allows the embedded type fresh sausage to have a maximum humidity of 70% (Brasil, 2000). Therefore, the sausages of marine catfish filets formulated in the present work are in accordance to the maximum permitted legislation.

In addition to the variation between the meat raw materials, embedded formulations and additions of external fat can also influence the final quantity of lipids. The sausages of marine catfish contained on average  $11.59 \pm 0.18\%$  lipids (Table 1). This value was less than the maximum allowed by Brazilian law, which is 30% for fresh type sausages (Brasil, 2000). The results of the present work were also in accordance with that in pasteurised sausages prepared with MSM obtained from Nile tilapia filleting waste (14.38%) (Dallabona et al., 2013), hybrid *Clarias* catfish sausages with addition of 10% of palm oil (10.62%) (Raksakulthai et al., 2004), and sausages

of Arabian Sea meagre (*Argyrosomus heinii*) (12.2%) (Al-Bulushi et al., 2013). Therefore, in addition to being close to what is observed in previous studies with embedded fish, the amount of lipids of sausages of marine catfish was also in accordance with the maximum allowed by Brazilian law (Brasil, 2000).

Fish meat has high amounts of protein, a good balance of essential amino acids, and high digestibility (Contreras-Guzmán, 2002). Thus, products produced with meat of fish also tend to have good quality protein. Sausages prepared with marine catfish meat presented on average  $18.98 \pm 0.47\%$  protein (Table 1) and were close to those prepared with meat from *Tetradon fahara* (18.61%) and *Clarias lazera* + *Tetradon fahara* (18.93%) (Ahmed and Elhaj, 2011), smoked sausages prepared with MSM obtained from Nile tilapia filleting waste (19.30%) (Dallabona et al., 2013), and sausages of *Lutjanus erythropterus* (19.7%) (Al-Bulushi et al., 2013). The Brazilian legislation (Brasil, 2000) allows the sausages of fresh type present at least 12% protein, so the sausages formulated in the present work were above the minimum required by the Brazilian law.

The sausages of marine catfish presented  $3.02 \pm 0.34\%$  ash (Table 1). The high value of ashes in the sausages might have occurred by adding the other ingredients in the formulation such as bacon, salt, flavouring and additives. Similar values were also found in sausages prepared with meat of *Clarias lazera* + *Tetradon fahara* (3.23%) (Ahmed and Elhaj, 2011), sausage of hybrid *Clarias* catfish (3.36%) (Raksakulthai et al., 2004) and sausages prepared with minced fish from Nile tilapia filleting waste (3.40%) (Oliveira Filho et al., 2010b). The Brazilian legislation (Brasil, 2000) does not prescribe the concentration of ash for embedded type fresh sausage, so there is no way to compare the composition with any set limits. However, the ashes are composed of minerals such as iron, zinc, and phosphorus, which are important in achieving a balanced diet.

The Brazilian legislation also does not prescribe the limit of carbohydrates in fresh sausage. The sausages formulated in the present work yielded  $6.76 \pm 0.54\%$  carbohydrates (Table 1), probably resulting from the addition of meat additives.

Table 1. Chemical-nutritional composition (mean  $\pm$  standard deviation) of fresh type sausages prepared with marine catfish fillets (*Sciades herzbergii*)

Component	(%)	Reference value (Brasil, 2000)
Moisture	$59.59 \pm 0.53$	70% Maximum
Lipids	$11.59 \pm 0.18$	30% Maximum
Protein	$18.98 \pm 0.47$	12% Minimum
Ashes	$3.02 \pm 0.18$	Undefined
Carbohydrates	$6.76 \pm 0.54$	Undefined

### Sensory evaluation

The colour of the sausages prepared with marine catfish fillets was scored 7 "liked moderately" (Table 2). The acceptability index (AI) of the colour of the sausages was 87%. As can be seen, the colour of the sausages were very well accepted by the panellists as according to Dutcosky (1996), values above 70% indicate the product's acceptance. This result is interesting because the formulated fish sausage was not a common product in Brazil, thus the panellists would have not been able to compare it with the colour of a commercial fish sausage. However, as the flesh of marine catfish has a reddish aspect, the panellists were probably not surprised by the colour because the traditional sausages made from beef generally have similar coloration.

Another interesting aspect is that because of the good acceptability of colour, it would not be necessary to use natural or artificial dyes, which can make the product healthier and less costly if it is produced on an industrial scale. The colour of the sausages formulated in the present work was better than the sausages of small scale mud carp (*Cirrhina microlepis*) of 5.3 "not liked nor disliked" to 6.9 "slightly liked" (Prabpree and Pongsawatmanit, 2011), sausages prepared with minced fish from Nile tilapia filleting waste of 4.1 "moderately disliked" to 6.1 "moderately liked" (Oliveira Filho *et al.*, 2010b), and close to that observed in the sausages prepared with MSM obtained from Nile tilapia filleting waste of 7.3 "moderately liked" (Dallabona *et al.*, 2013).

The odour of sausages of marine catfish formulated in the present work received a rating equivalent to "moderately liked" (Table 2). The odour of marine fish is usually stronger than freshwater fish. This happens because marine fish tend to concentrate more components of non-protein nitrogen that are volatile low-molecular-weight compounds such as peptides, free amino acids, nucleic acids, nitrates, amides, amines, and ammonia (Contreras-Guzmán, 2002). However, the good acceptance of the odour might be caused by the fact that the sausages were made with fresh fillets, thus retaining the good smell of sausages. In addition, added spices commonly used in the production of traditional fresh sausage also contributes to the characteristic smell of commercial sausage. The smell of the sausages formulated in the present work was almost similarly scored with sausages made from fresh trout fillets (score 7) (Dincer and Cakli, 2010), minced fish obtained from filleting waste of Nile tilapia (score 7.5) (Bartolomeu *et al.*, 2014) and pasteurised sausages prepared with MSM obtained from Nile tilapia filleting waste (score 7.5) (Dallabona *et al.*, 2013).

The texture of the marine catfish sausages formulated in the present work was scored as "very liked" (Table 2). This excellent acceptance of the sausage texture shows that the texture of the fillets, percentages of ingredients, and preparation conditions were adequate according to the standards of the panellists. The acceptance of the texture of fresh marine catfish type sausages formulated in the present work was better than that of sausages prepared with small scale mud carp, score 7.1 (Prabpree and Pongsawatmanit, 2011), bologna sausages of minced fish obtained from filleting waste of Nile tilapia, score 7.5 (Bartolomeu *et al.*, 2014) and pasteurised and smoked sausages prepared with MSM obtained from Nile tilapia filleting waste, scores 7.5 and 7.3, respectively (Dallabona *et al.*, 2013).

The flavour of the sausages of catfish scored the highest among other sensory attributes (Table 2). The *vongole* (*Anomalocardia brasiliiana*) sausages also scored "very liked" (Bispo *et al.*, 2004). This result was surprising because the marine catfish, on the coast of north eastern Brazil, are underutilised, consumed primarily by fishermen themselves. This highlights the importance of studies on the development of technological products using poorly-exploited species.

The overall acceptance followed all other sensory attributes (Table 2). However, the intent of purchase was scored  $4.4 \pm 0.7$  ("probably would buy"). These great sensory results and purchase plans show that the fresh type sausages prepared with meat of marine catfish are products with manufacturing and marketing potentials.

Table 2. Sensory evaluation (mean  $\pm$  standard deviation) and acceptability index of fresh type sausages prepared with marine catfish fillets (*Sciades herzbergii*)

Sensory attribute	Score <sup>1</sup>	Acceptability index (%)
Colour	7.8 $\pm$ 1.0	87.0%
Odour	7.4 $\pm$ 1.4	82.7%
Texture	8.0 $\pm$ 1.0	88.9%
Flavour	8.1 $\pm$ 1.0	90.2%
Overall Acceptance	8.0 $\pm$ 0.8	88.9%

<sup>1</sup>9-point hedonic scale: 1: disliked very much, 9: liked very much.

### Physico-chemical and microbiological stability

#### Lipid oxidation

The index of TBARS (thiobarbituric acid reactive substances) is widely used to indicate the degree of lipid oxidation in food products (Bartolomeu *et al.*, 2014; Ozpolat and Patir, 2016). It quantifies the quantity of malonaldehyde, one of the main products of decomposition of hydroperoxides of polyunsaturated fatty acids formed during the oxidative process of lipids of fish meat (Ribeiro *et*

Table 3. Adjusted model, coefficient of determination adjusted ( $R^2$ ) and p-value of the first or second order regressions of the dependent variables of fresh type sausages made with marine catfish fillets (*Sciades herzbergii*) stored for 32 days under refrigeration (6°C) and four months under freezing conditions (-20°C).

Variable	Storage conditions	Adjusted Model	R2 adjusted	p-value
TBARS (mg malonaldehyde/kg)	Refrigeration	$y = -0.0013x^2 + 0.0601x + 0.3552$	0.7683	$6.14e^{-05}$
	Freezing	$y = -0.0234x^2 + 0.157x + 0.451$	0.8659	$2.31e^{-06}$
TVB-N (mg N/100g)	Refrigeration	$y = 0.0127x^2 + 0.5533x + 12.442$	0.9603	$1.562e^{-09}$
	Freezing	$y = 1.0763x + 12.22$	0.2903	0.022280
WHC (%)	Refrigeration	$y = 0.0261x^2 - 1.2044x + 86.779$	0.7203	0.00019
	Freezing	-	-	>0.05
pH	Refrigeration	$y = 0.002x^2 - 0.075x + 6.6881$	0.7046	$2.63e^{-04}$
	Freezing	-	-	>0.05
Instrumental Colour				
$L^*$	Refrigeration	-	-	>0.05
	Freezing	$y = 0.3865x^2 - 2.9655x + 62.87$	0.4726	0.0085
$a^*$	Refrigeration	$y = 0.0064x^2 - 0.2013x + 8.0177$	0.5426	0.0036
	Freezing	$y = -0.1515x^2 - 0.1862x + 7.7005$	0.9115	0.0001908
$b^*$	Refrigeration	-	-	>0.05
	Freezing	$y = 0.2687x^2 - 1.8058x + 10.708$	0.3164	0.040

al., 2013). Lipid oxidation is responsible for a series of changes that lead to the loss of nutritional value, rejection of the product, and the formation of toxic compounds. Some authors suggest that values above 3 mg malonaldehyde/kg in fish products might be potentially harmful to consumers' health as well as noticeable in sensory analysis (Dallabona et al., 2013; Bartolomeu et al., 2014).

For sausages of marine catfish stored under refrigeration, the values of TBARS, estimated by the equation of second degree, significantly increased ( $p < 0.05$ ) from 0.57 mg malonaldehyde/kg (four days of storage) to 1.05 mg malonaldehyde/kg between days 22 to 25 with a slight decrease by the end of 32 days of storage (0.95 mg malonaldehyde/kg) (Table 3). Corroborating with the result, other studies also noted increased values of TBARS in refrigerated fish sausages. For example, sausages prepared from three freshwater fish species subjected to different methods of smoking increased from 0.73 to 0.98 mg malonaldehyde/kg to 1.5 to 2.5 mg malonaldehyde/kg after 42 days of storage at 4°C (Ozpolat and Patir, 2016). The sausages prepared with minced fish from Nile tilapia filleting waste also showed an increase in TBARS index of 0.75 to 1.08 mg malonaldehyde/kg for 42 days at 0°C (Oliveira Filho et al., 2010a), and sausages prepared with rohu fish (*Labeo rohita*) for 13 days of storage at 5°C from 0.1 to 0.4 mg malonaldehyde/kg (Sini et al., 2008). According to Oliveira Filho et al. (2010a), lipid oxidation in fish sausages might be related to the amount of lipids, type of fatty acid, degree of grinding of fillet, and

the presence of oxygen. Therefore, the inclusion of swine fat and grinding of catfish fillets might have facilitated the lipid oxidation of the sausages in the present work. However, Brazilian legislation does not prescribe a maximum amount of lipid oxidation in fish or fish products, although toxic components such as aldehydes, ketones, alcohols, acids and hydrocarbons are formed when the food is highly oxidised (Oliveira Filho et al., 2010a).

In sausages stored under freezing (-20°C), there was an evolution ( $p < 0.05$ ) in TBARS values of 0.45 mg malonaldehyde/kg reaching the apex after three months (0.71 mg malonaldehyde/kg) and then a slight decrease after four months (0.70 mg malonaldehyde/kg) (Table 3). The commercial sausages of fillets of *Lutjanus erythropterus* and fillets of *Argyrosomus heinii* added with spices also showed an increase in the lipid oxidation under frozen storage (Al Bulushi et al., 2013), thus agreeing with the results observed in the present work.

The range of TBARS variation of the frozen catfish sausages was lower than those refrigerated, and the variation between the lowest and the highest value was 0.26 mg malonaldehyde/kg in frozen sausages and 0.48 mg malonaldehyde/kg in refrigerated sausages. In addition, at the point of initial analysis (four days storage), the TBARS value of frozen stored sausages was lower, indicating that the lipid oxidation started faster at higher storage temperature. Therefore, according to the TBARS analysis, chilled catfish sausages could be consumed for up to 32 days, and frozen for up to four months.

### Total Volatile Bases Nitrogen (TVB-N)

The TVB-N corresponds to ammonia, dimethyl amine and trimethyl amine, all of which are formed by the nucleotide breakdown and amino acid deamination by bacterial enzymes (Oliveira Filho *et al.*, 2010a). The TVB-N of marine catfish sausages stored under cooling significantly increased ( $p < 0.05$ ) from 14.9 mg N/100 g after four days storage to 43.1 mg N/100 g at the end of 32 days storage (Table 3), i.e., an increase of 28.3 mg N/100 g. In other studies of fish sausages, an increase in TVB-N values was also observed (Raju *et al.*, 2003; Sini *et al.*, 2008). Brazilian legislation allows a maximum of 30 mg N/100 g of TVB-N for fish to be deemed fit for human consumption (Brasil, 2000). When compared to chilled stored catfish sausages, it was observed that, according to the model of a significant second equation, the maximum allowed by the legislation was reached after 21 days at 29.7 mg N/100 g of VNB.

There was also a significant increase ( $p < 0.05$ ) in TVB-N values from 12.2 mg N/100 g (time 0) to 16.5 mg N/100 g (four months of frozen storage) (Table 3). Despite this, the total increment was only 4.3 mg N/100 g. Sausages made from *vongole* also showed little elevation in TVB-N values during 90 days of frozen storage (Bispo *et al.*, 2004). This could have been due to the slow action of the endogenous enzymes and the non-development of spoilage bacteria under freezing conditions. In addition, the catfish sausages stored frozen did not reach the maximum of TVB-N allowed by the Brazilian legislation for fish (30 mg N/100 g) (Brasil, 2000), which could suggest appropriate consumption, according to this analysis, during four months of frozen storage.

### Water Holding Capacity (WHC)

Water holding capacity (WHC) analysis indicates how much fish muscle or fish meat products could retain water when subjected to an external agent such as centrifugation, pressure or heating (Dincer and Cakli, 2010; Sleder *et al.*, 2015). This analysis is closely related to the degree of denaturation of myofibrillar proteins (myosin and actin) that has influence on the physical aspects of colour, texture and sensorial acceptance of fish (Viegas *et al.*, 2012).

It was observed that the WHC of refrigerated marine catfish sausages significantly decreased ( $p < 0.05$ ) from 82.4 to 72.9% after 22 days, and a small increase to 75.0% after 32 days of storage (Table 3). This might indicate that under refrigeration, the myofibrillar proteins of marine catfish sausages underwent denaturation until approximately 22 days of storage with some stability thereafter. Sausages

made with tambaqui fish (*Colossoma macropomum*) added with different concentrations of pork fat showed WHC between 71.83 and 74.71% (Sleder *et al.*, 2015), which is almost similar to the values found in the present work. However, in sausages made from small scale mud carp (Prabpree and Pongsawatmanit, 2011) or with rainbow trout (*Onchorynchus mykiss*) (Dincer and Cakli, 2010) the WHC values were above 90%. This difference might have occurred because of variations in the intrinsic meat characteristics of fish species, the types and proportions of ingredients, and the additives in the formulations.

There was no significant difference ( $p > 0.05$ ) in WHC over four months frozen storage of marine catfish sausages, presenting an average of  $85.0 \pm 0.9\%$ . This result might indicate that the best preservation method could be freezing in comparison with refrigeration. However, the WHC of the sausages formulated in the present work were lower than that observed in rainbow trout fillet sausages during 14 days of frozen storage, presenting values between 96.22 and 98.51% (Dincer and Cakli, 2010), which might have occurred due to variations in the formulations between the sausages and sausage types.

### pH measurement

The pH of refrigerated sausages presented a second degree variation ( $p < 0.05$ ) during the 32 days storage period (Table 3). The sausages started with pH 6.4 (four days of storage), decreasing to 6.0 from 14 to 24 days and increasing to 6.3 at the end of 32 days of storage. The decrease in pH might be due to the action of lactic acid bacteria, usually found in vacuum-packed meat sausages, and storage under refrigeration (Dallabona *et al.*, 2013). The increase in pH after 24 days might have occurred with the development of other types of bacteria, competing with the lactic acid bacteria, thereby rising the pH of the sausages. In sausages made from Japanese threadfin bream (*Nemipterus japonicus*), there was also a decrease in pH from 6.75 to 6.19 after 30 days of storage at 6°C (Raju *et al.*, 2003). For fish meat to be fit for human consumption, the pH must be below 6.8 (Oliveira Filho *et al.*, 2015). Therefore, according to this analysis, the sausages would be fit for consumption during the evaluated time period.

The frozen marine catfish sausages did not present a significant difference ( $p > 0.05$ ) in pH over four months of storage, presenting a general average of  $6.1 \pm 0.1$ . This result agrees with that observed in sausages made with *vongole* during 90 days of frozen storage, presenting values between 5.20 to 5.27 (Bispo *et al.*, 2004).

Table 4. Microbiological evaluation (mean  $\pm$  standard deviation) of fresh type sausages prepared with marine catfish fillets (*Sciades herzbergii*) stored for 32 days under refrigeration (6°C)<sup>1,2</sup>

Microbiology	Storage time (days)				
	4	11	18	25	32
<i>E. coli</i> (log CFU/g)	< 2 <sup>b</sup>	< 2 <sup>b</sup>	< 2 <sup>b</sup>	< 2 <sup>b</sup>	3.7 $\pm$ 0.1 <sup>a</sup>
<i>Salmonella</i> sp. (25 g)	Absent	Absent	Absent	Absent	Absent
<i>Staphylococcus</i> positive coagulase (log CFU/g)	< 2 <sup>a</sup>				
Psychrotrophic aerobic (log CFU/g)	4.6 $\pm$ 0.0 <sup>a</sup>	3.2 $\pm$ 0.1 <sup>b</sup>	2.6 $\pm$ 0.1 <sup>d</sup>	2.9 $\pm$ 0.0 <sup>c</sup>	4.6 $\pm$ 0.1 <sup>a</sup>

<sup>1</sup>Different letters on the same line indicate significant difference ( $p < 0.05$ ).

<sup>2</sup>< 2 log CFU/g is the minimum detection limit of the Compact Dry<sup>®</sup> kits used for analysis.

### Instrumental colour

Food colour is one of the main factors that influence the consumers' perception and acceptance (Bartolomeu *et al.*, 2014). Lightness ( $L^*$  value) of sausages made with marine catfish meat showed no significant difference ( $p > 0.05$ ) during 32 days of refrigeration storage, presenting a mean of  $61.7 \pm 1.8$ . In other studies that evaluate the lightness of fish sausages, similar behaviour was also observed (Oliveira Filho *et al.*, 2010a; Bartolomeu *et al.*, 2014; Tirloni *et al.*, 2015). This result is interesting because the TBARS analysis indicated a high lipid oxidation (Table 3), but apparently did not influence the lightness of the refrigerated sausages. Differently, in frozen sausages, variation in  $L^*$  values occurred, with a decrease from 62.9 at time 0 to 56.8 after four months of storage (Table 3). No previous reports have evaluated the lightness ( $L^*$  value) of fish sausages stored under freezing.

The redness ( $a^*$ ) of the refrigerated marine catfish sausages showed a quadratic variation ( $p < 0.05$ ) over the 32 days storage period, decreasing from 7.3 to 6.4 up to 17 days and increasing thereafter to reach a final value of 8.1 (Table 3). Bologna sausages manufactured with Nile tilapia also showed variation in redness ( $a^*$ ) for 30 days of storage at 6°C (Bartolomeu *et al.*, 2014). In the frozen catfish sausages, the redness ( $a^*$ ) significantly decreased ( $p < 0.05$ ) over time, with values varying from 7.7 to 4.5 (Table 3). It was observed that freezing catfish sausages yielded a more pronounced decrease in redness as compared to refrigerating.

The yellowness ( $b^*$ ) did not change ( $p > 0.05$ ) in the refrigerated sausages stored for 32 days, presenting a general mean of  $10.6 \pm 0.8$ . When compared with the data from the literature, sausages made from meagre (*Argyrosomus regius*) presented values (8.56 to 9.21) close to that of the present work (Ribeiro *et al.*, 2013). Sausages made with Atlantic salmon trimming (Tirloni *et al.*, 2015), pasteurised and smoked sausages prepared with MSM obtained from Nile tilapia filleting waste (Dallabona *et al.*,

2013), rainbow trout sausages with fresh or frozen fillet (Dincer and Cakli, 2010), bologna sausage made from Nile tilapia (Bartolomeu *et al.*, 2014), meagre sausage (Ribeiro *et al.*, 2013) and sausages prepared with minced fish from Nile tilapia filleting waste (Oliveira Filho *et al.*, 2010a) also did not present variation in the yellowness ( $b^*$ ) when stored under refrigeration.

In the sausages kept under freezing, the yellowness ( $b^*$ ) significantly decreased ( $p < 0.05$ ), ranging from 10.7 (time 0) to 7.8 (four months) (Table 3). The yellowness in the refrigerated sausages were similar to those observed at point 0 of the frozen stored sausages. This suggests that the four months freezing rendered the sausages less yellowish.

### Microbiological analyses

The refrigerated marine catfish sausages presented a thermotolerant coliform (*E. coli*) count of < log 2 CFU/g up to 25 days of storage, where from this point (32 days storage) of the analysis showed the presence of this bacterium (Table 4). This result shows that although the product was prepared under adequate hygienic conditions, at the final moment of storage they began to develop faster, inferring that this evaluation point initially defined the end of the lag phase. Resolution 12 of January 2, 2001, from the National Agency for Sanitary Surveillance (ANVISA, 2001) comments that chilled or frozen stored fish products may contain a maximum of  $10^3$  CFU/g (log 3 CFU/g) of thermotolerant coliforms (*E. coli*). Therefore, catfish sausages at 32 days of refrigerated storage were already above the maximum allowed, and consumption of this food up to 25 days of storage is recommended. Fresh sausages made with tambaqui fish also had low counts (log 1.2 to 2.2 CFU/g) of thermotolerant coliforms, when stored for up to 12 days under refrigeration (Sleder *et al.*, 2015).

The presence of *Staphylococcus* positive coagulase bacteria was < log 2 CFU/g up to 32 days of storage of marine catfish sausages when kept under

Table 5. Microbiological evaluation (mean  $\pm$  standard deviation) of fresh type sausages prepared with marine catfish fillets (*Sciades herzbergii*) stored for four months under freezing conditions ( $-20^{\circ}\text{C}$ )<sup>1,2</sup>

Microbiology	Storage time (months)				
	0	1	2	3	4
<i>E. coli</i> (log CFU/g)	< 2	< 2	< 2	< 2	< 2
<i>Salmonella</i> (25 g)	Absent	Absent	Absent	Absent	Absent
<i>Staphylococcus</i> positive coagulase (log CFU/g)	< 2	< 2	< 2	< 2	< 2
Psychrotrophic aerobic (log CFU/g)	4.6 $\pm$ 0.0 <sup>a</sup>	4.2 $\pm$ 0.0 <sup>b</sup>	3.4 $\pm$ 0.0 <sup>d</sup>	3.9 $\pm$ 0.0 <sup>c</sup>	3.9 $\pm$ 0.0 <sup>c</sup>

<sup>1</sup>Different letters on the same line indicate a significant difference ( $p < 0.05$ ).

<sup>2</sup>< 2 log CFU/g is the minimum detection limit of the Compact Dry<sup>®</sup> kits used for analysis.

refrigeration (Table 4). ANVISA (2001) allows up to  $10^3$  CFU/g (log 3 CFU/g) of *Staphylococcus* positive coagulase in fish products. This shows that besides the processing of the sausages being carried out in a hygienic way, the packaging and storage temperature could also be efficient to conserve and maintain quality, in accordance with what determined by the legislation. Sausages made from meat of *Clarias lazera* or *Tetradon fahaka* were also imperceptible in the *Staphylococcus* counts during 30 days of storage at  $5^{\circ}\text{C}$  (Ahmed and Elhaj, 2011). The *Salmonella* sp. was absent in the sausages at all evaluated time periods. In other types of fish sausages there was also no presence of *Salmonella* sp. (Oliveira Filho *et al.*, 2010a; Ahmed and Elhaj, 2011).

The total count of the psychrotrophic aerobic bacteria presented interesting behaviour, because initially they were present a high count, decreasing throughout the storage period and returning to a high count at the final point of analysis (Table 4). This phenomenon could be explained by the fact that initially the bacteria were present in the food, and with the passage of time the additives used in the preparation of the sausages, as for example the curing salt, the low temperature and the vacuum packaging, caused the decrease of the psychrotrophic aerobic bacteria. However, over time all conservation aids appear to lose their efficacy, and bacteria have adapted and restarted the cell multiplication, reaching values close to that observed in the initial evaluation. In Brazil, the legislation does not prescribe the maximum amount for aerobic psychrotrophic bacteria for refrigeration or frozen stored fish products. Despite this, the food industry uses as maximum limit of microbial contamination at log 6 CFU/g for this food group (Tirloni *et al.*, 2015).

Therefore, as stipulated by the industry, all catfish sausages would be fit for consumption until the end of 32 days of storage. Similar results of catfish sausages were observed in sausages made from meat

of *Clarias lazera* or *Tetradon fahaka* which presented a count between log 3 and 5 CFU/g of total counts of aerobic psychrotrophic bacteria during 30 days of storage at  $5^{\circ}\text{C}$  (Ahmed and Elhaj, 2011).

The frozen marine catfish meat sausages had <log 2 CFU/g of thermotolerant coliforms (*E. coli*) and were absent from *Salmonella* sp. up to four months of storage (Table 5). In agreement with that observed in the present work, *vongole* sausages also did not present counts of thermotolerant coliforms and were absent from *Salmonella* sp. when stored for up to three months under freezing (Bispo *et al.*, 2004). The count of *Staphylococcus* coagulase positive in the sausages was undetectable (<log 2 CFU/g) for four months under frozen storage (Table 5). ANVISA (2001) prescribes the maximum allowable limit of this bacterium in frozen or frozen stored fish products at log 3 CFU/g; so the formulated sausages could be consumed when kept for up to four months of frozen storage.

The total count of aerobic psychrotrophic bacteria from marine catfish sausages decreased throughout the frozen storage (Table 5). This phenomenon was expected, because most of the pathogenic bacteria cannot tolerate very low temperatures, and a small decrease in the count of this type of bacteria is common in frozen products. As observed in the present work, commercial sausages from fillets of *Lutjanus erythropterus* and experimental sausages from fillets of Arabian Sea meagre also decreased in the total count of psychrotrophic bacteria from log 3.5 to 2.8 CFU/g (experimental sausages) and log 5.1 to 4.6 CFU/g (commercial sausages) after three months of frozen storage (Al-Bulushi *et al.*, 2013). If the comparison is made against the maximum allowable limit followed by the food industry (log 6 CFU/g; Tirloni *et al.*, 2015), the catfish sausages might be consumed for up to four months of frozen storage.

## Conclusion

The production of fresh sausages using marine catfish fillets (*Sciades herzbergii*) is a good way to add value to a species of low commercial value captured along the northeast coast of Brazil, due to the good nutritional quality, sensorial acceptance, and shelf life between 21 and 25 days when kept under refrigeration at 6°C and at least four months under freezing at -20°C.

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