










Conservation and quality of grumatã (*Prochilodus lineatus*) fillets after different depuration periods and frozen storage

Conservação e qualidade de filés de grumatã (*Prochilodus lineatus*) após diferentes períodos de depuração e congelamento

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Abstract

The aim of this study was to evaluate the physicochemical, microbiological, sensory and meat quality characteristics of grumatã fillets after different depuration (0, 48, 72 and 96 h) periods and frozen storage (0, 2, 4 and 6 months). The fish collected in a dam were distributed in tanks at a density of 3.8 kg m⁻³. After depuration periods, all fish were filleted and the fillet samples stored (-18 °C) until analyses. Lower fat content was found in fish submitted to depuration for 48 and 96 h compared to the non-depurated and those depurated for 72 h. Muscle protein was preserved in 48 and 96 h treatments. Coagulase-positive *Staphylococcus*, coliforms at 45 °C and *Salmonella* spp. were not observed in the fillets, but aerobic mesophilic microorganisms and coliforms at 35 °C were detected. Sensory analysis showed no significant differences in appearance, colour, texture, flavour attributes and overall acceptance. During frozen storage, pH, total volatile basic nitrogen (TVB-N), peroxide and thiobarbituric acid reactive substance (TBARS) values were evaluated in fillet samples every two months. The pH values increased up to four months of storage. The TVB-N values ranged in fillets from fish depurated for 48 and 96 h. Peroxide values increased from the fourth month of storage, with the highest values observed in the sixth month. For TBARS values, increased values were found in fillets from non-depurated fish and lower values in other depuration periods. Thus, depuration is more efficient in maintaining the quality of grumatã fillets in frozen storage. **Keywords:** Off-flavor; Flavor; Odor; Lipid oxidation; Sensory analysis.

Resumo

O objetivo deste estudo foi avaliar as características físico-químicas, microbiológicas, sensoriais e de qualidade de carne de filés de grumatã após diferentes períodos de depuração (0, 48, 72 e 96 h) e congelamento (0, 2, 4 e 6 meses). Os peixes coletados em barragem foram distribuídos em tanques em uma densidade de 3,8 Kg m⁻³. Após os períodos

de depuração todos os peixes foram filetados e as amostras de filé foram estocadas (-18°C) até serem analisadas. Menor conteúdo de gordura foi encontrado nos peixes submetidos a depuração por 48 e 96 h comparado aqueles não depurados e depurados por 72 h. Proteína muscular foi preservada nos tratamentos 48 e 96 h. *Staphylococcus* coagulase-positiva, coliformes a 45°C e *Salmonella* spp. não foram observados nos filés, mas microorganismos mesófilos aeróbios e coliformes a 35°C foram encontrados. A análise sensorial não demonstrou diferenças significativas nos atributos aparência, cor, textura, sabor e aceitação global. Durante o congelamento, os valores de pH, Bases Nitrogenadas Voláteis Totais (BVNT), peróxidos e Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS) foram avaliados em amostras de filé a cada dois meses. Os valores de pH aumentaram após quatro meses de estocagem. Os valores de BVNT variaram nos filés dos peixes depurados por 48 e 96 h. Os valores de peróxidos aumentaram a partir do quarto mês de congelamento e no sexto mês os valores mais altos foram observados. Para TBARS, valores altos foram encontrados em filés de peixes não depurados e menores valores nos tratamentos depurados. Assim, a depuração mostrou ser mais eficiente para manter a qualidade de filés de grumatã armazenados congelados.

Palavras-chave: *Off-flavor*; Sabor; Odor; Oxidação lipídica; Análise sensorial.

Introduction

In Brazil, the growth of aquaculture is related to the demand for healthy foods, since fish meat has a high content of essential amino acids, high digestibility, polyunsaturated fatty acids and low cholesterol⁽¹⁾. However, the availability of a fish species does not represent high acceptance and consumption by consumers. An example is grumatã or curimatã (*Prochilodus lineatus*), one of the species most commonly found and fished in Brazilian rivers⁽²⁾. Due to the presence of intramuscular spines, an unpleasant meat flavour and an undesirable meat odour, there is low acceptance by consumers.

Due to its detritivorous-iliophagous eating habit, the grumatã is known as 'ground porridge'. Bacteria and fungi are part of its diet and responsible for geosmin and 2-methylisoborneol (MIB) production, compounds that cause a muddy and musty flavour, also known as an off-flavour. Geosmin and MIB are absorbed through the gills, intestines or skin of fish and can be accumulated in fat tissues⁽³⁾. The grumatã can be classified as a moderately fat species, due to the presence of fat ranging from 5–10%. This content can contribute to the muddy and musty flavour and undesirable odour from the meat⁽⁴⁾. As an alternative to soften the off-flavour, a depuration process can be applied, a step where fish are fasted in tanks with clean and running water before slaughter.

Although the fish goes through depuration periods, a series of physical, chemical, biochemical and microbiological changes occur after slaughter. These changes start

with endogenous enzymatic action in the muscles (autolysis), which is associated with intrinsic factors that determine the fish deterioration, such as the presence of a high amount of water and nutrients in the meat, unsaturated lipids, a pH close to neutrality and activity of microorganisms⁽¹⁾. In addition, the high degree of unsaturation of fatty acids in fish meat is responsible for the susceptibility to lipid oxidation. Therefore, during fish storage, analyses must be carried out to monitor and assess the quality. Thus, the objective of this study was to evaluate physicochemical, microbiological, sensory and meat quality characteristics of grumatã after different depuration periods (0, 48, 72 and 96 h) and frozen storage (0, 2, 4 and 6 months).

Material and methods

The licence to collect wild animals was granted through the Chico Mendes Institute for Biodiversity Conservation (protocol 47353/2). All procedures that involved animals, meat, and sensory analysis were approved by the Research Ethics Committee of the Federal University of Pampa (number 1.013.479, CAAE protocol number 39982314.0.0000.5323).

The grumatã (*Prochilodus lineatus*) were collected in a dam located in the municipality of Uruguaiana, Rio Grande do Sul state, Brazil (29° 34' 47.3" S; 56° 50' 39.1" W), outside the reproductive period of the species. The fish were captured by a 7 cm mesh net and transported (transport box capacity 250 L) to the Fisheries and Aquaculture Technology Centre at the Federal University of Pampa. Twenty-one fish (males and females) were weighed (1.89 ± 0.47 kg) and measured (45.22 ± 2.39 cm) individually. Of these, six specimens were slaughtered immediately, to constitute the 0 h treatment (without depuration). The other 15 specimens were distributed at random into three tanks, with approximately 2100 L (five fish per tank), with a continuous water flow, and a biomass remaining close to 3.8 kg m^{-3} for the depuration step. The depuration periods were 48 h, 72 h and 96 h. In the present study, the 24 h depuration period was not reported due to our previous results⁽⁴⁾ showing no differences in the sensory characteristics of the grumatã fillets, which can also be considered a very short period for depuration.

During depuration, water quality parameters of the tanks were analysed daily, obtaining the following average values: flow rate: $4.67 \pm 0.9 \text{ L min}^{-1}$; temperature: 23.53 ± 0.37 °C; dissolved oxygen: $5.38 \pm 1.22 \text{ mg L}^{-1}$ and total ammonia: $0.05933 \pm 0.026 \text{ mg L}^{-1}$. At the end of the period, alkalinity, hardness, pH, conductivity and turbidity were also evaluated. The average values found were: 201.67, 121.33 $\text{mg L}^{-1} \text{ CaCO}_3$, 7.74 units, 661.367 $\mu\text{S cm}^{-1}$ and 0 nephelometric turbidity units, respectively.

All animals were slaughtered by hypothermia (water: ice 1:1) and weighed to calculate weight loss (%). Fillets were removed and weighed in order to determine the fillet yield (%). Fillets were washed in running water and after, in water containing 10 ppm of chlorine. The fillet samples were stored in plastic bags at -18 °C for 30 days until used for evaluating the physicochemical and microbiological composition, and sensory analysis. Sub-samples were also stored at -18 °C for measuring the conservation of the fillet at zero (initial), two, four and six months of storage.

The physicochemical analysis as dry matter (105 °C/24 h), ash (500 °C/4 h) and crude protein (N × 6.25 by the micro-Kjeldahl method) were determined according to the methods proposed by the Association of Official Analytical Chemists⁽⁵⁾. The fat was extracted and quantified by the cold extraction method proposed by Bligh and Dyer⁽⁶⁾.

The microbiological quality of the fillets was evaluated according to the methodologies proposed by the Brazilian Ministry of Agriculture, Livestock and Supply⁽⁷⁾. Then, the amount of coliforms at 35 °C and 45 °C, coagulase-positive *Staphylococcus*, *Salmonella* spp., aerobic mesophilic microorganisms, and moulds and yeasts were determined.

For the sensory analysis, a total of 73 untrained consumers aged 18–60 years (52% male and 48% female) evaluated the samples. Approximately 10 g of fillet samples were wrapped in aluminium foil, cooked in an electric oven (250 °C for 15 min), and immediately offered to the consumers. Water and water and salt biscuits were supplied to cleanse the palate between samples. Consumer acceptance was performed using a seven-point hedonic scale, ranging from 1 = totally dislike to 7 = totally like. Each consumer was asked to evaluate appearance, colour, texture, flavour and overall acceptance of the fillet samples, as proposed by Dutcosky⁽⁸⁾.

To evaluate the meat quality, the samples stored at –18 °C were analysed at zero, two, four and six months of storage. The pH (hydrogen potential) was measured using a portable meat pH metre (Akso Eletronic Products, Rio Grande do Sul, Brazil). To determine total volatile nitrogenous bases (TVB-N)⁽⁹⁾, 50 g of fillet samples were homogenised in 5% trichloroacetic acid solution (1:3 w:v) for 1 min. Afterwards, 10 mL of filtrate was transferred to a distillation tube and 1 g of magnesium oxide and 20 mL of water were added. The distilled product (100 mL) was received in a solution of boric acid and mixed indicator and titrated with 0.01 N sulfuric acid solution for the determination of ammonia and volatile amines.

Thiobarbituric acid reactive substances (TBARS) were evaluated according to Buege and Aust⁽¹⁰⁾. For this analysis, a sample (1 g) was homogenised in 1.15% potassium chloride solution (1:5 w:v) and then centrifuged (10 min at 3,000 rpm). The supernatant (0.75 mL) was incubated in a water bath (100 °C/15 min) with 30% trichloroacetic acid solution and 0.67% thiobarbituric acid. After cooling, n-butyl alcohol (1.5 mL) was added to extract the coloured product. The absorbance was measured at 535 nm. The standard curve was constructed using malonyldialdehyde (MDA) solution (0.6 to 12 nmol).

Peroxides were determined according to Chapman and Mackay⁽¹¹⁾. Initially, fat was extracted from fillet samples⁽⁶⁾. Then, fat samples (200 µL) were dissolved in benzene:methanol solution (70:30 v:v), followed by the addition of 30% ammonium thiocyanate (10 µL) and ferrous chloride (10 µL). The samples were incubated at 50 °C for 2 min and assessed at 520 nm. The standard curve was constructed using iron solution (0.7 to 7.1 µmol).

In relation to the statistical analysis, data from the physicochemical and sensory analyses were analysed by one-way analysis of variance (ANOVA). The mean values were compared by Tukey's test ($p < 0.05$). Data for meat quality were analysed using two-way ANOVA (depuration period and months of storage). Means were compared

using Tukey's test ($p < 0.05$). Statistica software, version 7.0⁽¹²⁾ was used to perform the statistical analyses.

Results and Discussion

Data from the weight loss and fillet yield are shown in Table 1. All fish that were depurated had weight loss. This behaviour is related to the reduction in intestinal content and, probably, the body fat, which can influence the fillet yield. Regarding the fillet yield, the non-depurated fish yielded 45.8% and the depurated fish ranged from 36.5 to 40%. Due to the fusiform shape of its body, the grumatã has higher fillet yield compared to other species, such as tilapia, which has a compressed body and fillet yield ranging from 28 to 31%⁽¹³⁾. In addition, fillet yield is also related to the applied filleting method and the filler's skill.

Table 1. Weight loss, fillet yield, and physicochemical composition of fillets from *Prochilodus lineatus* after different depuration periods

Parameters (%)	Depuration (hours)			
	0	48	72	96
Weight loss	-	6.94 ± 3.42	7.17 ± 2.59	9.22 ± 2.67
Fillet yield	45.82 ± 2.25 ^b	36.53 ± 2.61 ^a	40.16 ± 2.57 ^a	37.40 ± 3.08 ^a
Dry matter	30.93 ± 4.12 ^{bc}	25.79 ± 1.04 ^a	33.22 ± 3.03 ^c	28.66 ± 1.03 ^{ab}
Ash*	3.57 ± 0.46 ^a	4.66 ± 0.40 ^b	3.27 ± 0.29 ^a	4.27 ± 0.33 ^b
Crude protein*	58.39 ± 2.66 ^a	77.66 ± 5.27 ^c	56.34 ± 2.45 ^a	66.03 ± 6.09 ^b
Fat*	19.20 ± 7.78 ^b	12.19 ± 2.60 ^a	23.95 ± 2.92 ^c	14.56 ± 4.18 ^a

Results are expressed as mean ± standard deviation (n = 4). *Results are expressed as percentage of dry matter. Different letters in the same row indicate significant differences between treatments by Tukey's test ($p < 0.05$).

For the physicochemical composition (Table 1), the fish submitted to 48 and 96 h of depuration had a lower dry matter (DM) content than those depurated for 72 h. In these treatments, higher ash and crude protein content and lower fat content were also found compared to the other treatments (0 and 72 h). In fillets from specimens of *P. cearensis* captured in dams from March to July (average weight: 400 g), values of 23.7% of DM, 5.4% of ash, 78% of crude protein and 16% of fat were reported (data on a DM basis)⁽¹⁴⁾. These values are similar to those found in the grumatã fillets depurated for 48 and 96 h. However, fat and DM (moisture) are the components most prone to changes in meat. The moisture and fat vary according to the diet fed, fish age and size, sex, reproductive stage, part of body analysed, time of the year and water temperature⁽¹⁵⁾. The specimens in this study were captured from a dam, a lentic environment with high food availability,

and were in the adult stage, factors that contributed to the high accumulation of body fat, since the values found in this study ranged from 3 to 7.8%, on a wet basis, or 12.1 to 23.9% of DM.

In addition to natural variations in meat composition between different specimens during depuration periods, changes observed in fat and crude protein contents of fillets can also be related to the use of body constituents, such as glycogen, protein and triglycerides, which provide an energy supply during periods of fasting. In the first hours, in a situation of food deprivation, hepatic glycogen is degraded to release glucose to body tissues. In the presence of depleted glycogen reserves, the liver starts to adopt the gluconeogenesis pathway, converting the precursor substrates – amino acids (degraded from muscle protein), glycerol (oxidation of triglycerides from adipose tissue) and lactate (anaerobic metabolism of red blood cells) – for conversion to glucose⁽¹⁶⁾. In longer periods (3 to 4 days of fasting), muscle proteolysis that provides amino acids for gluconeogenesis decreases because the protein reserve is limited. In this condition, fatty acids mobilised from adipose tissue are used in the synthesis of ketone bodies and represent an alternative energy to glucose for extra-hepatic tissues, such as nerve cells in the central nervous system and muscle cells⁽¹⁷⁾. Thus, in the present study, these metabolic routes may have also influenced the protein and fat content of meat, in fish that remained fasting during depuration periods (mainly to 48 and 96 h).

Microbiological analysis showed the presence of aerobic mesophilic microorganisms in the fillet samples (CFU g⁻¹): 0 h: 1.3 x 10³; 48 h: 1.0 x 10³; 72 h: 1.4 x 10³ and 96 h: 3.0 x 10³. Coliforms at 35 °C were also detected in all treatments (MPN g⁻¹): 0 h: 2.3 x 10¹; 48 h: 4.6 x 10²; 72 h: 1.1 x 10³ and 96 h: 4.6 x 10². The count of moulds and yeasts, coagulase-positive *Staphylococcus*, coliforms at 45 °C and *Salmonella* spp. was not observed in the fillet samples. According to the RDC n. 12 of the National Health Surveillance Agency (ANVISA)⁽¹⁸⁾, the mandatory microbiological analyses for the evaluation of fresh fish requires determining coagulase-positive *Staphylococcus* and *Salmonella* spp., in order to ensure the sanitary conditions of this product. Thus, the results obtained here were in accordance with the values established in the RDC n.12. According to Brazilian law, analyses of aerobic mesophilic microorganisms and coliforms at 35 °C are not mandatory, but their determination is important to indicate the effectiveness of good manufacturing practices (cleaning, disinfection and temperature control). The microorganisms detected here (low counts) must be eliminated by cooking processes applied before sensory analysis.

Concerning appearance, colour, texture and flavour attributes and overall acceptance, no significant differences were found in the grumatã fillets depurated for different periods (Table 2). For all attributes from different depuration periods, scores were 'like moderately'. These results show that the grumatã meat had high acceptance by untrained consumers.

In a study with specimens of grumatã collected in a dam and depurated for up to 120 h⁽⁴⁾, high acceptance for all attributes was reported and scores were 'neither like or dislike' and 'like moderately'. However, consumer acceptance of fillet samples was higher for the fish depurated for 96 h (15%) compared to non-depurated. Consumer acceptance

depends on the sensitivity of each individual and is influenced by cultural habits and standards. Furthermore, the elimination of off-flavour substances during depuration depends on the initial intensity of deposition of these molecules in adipose tissue and, consequently, on the fish's fat content⁽³⁾. Thus, the grumatã, characterised as 'moderately fat', tends to deposit more geosmin and MIB than 'lean' species, such as tilapia and pirarucu⁽¹⁹⁾. In this way, elimination of these molecules should take longer than the periods evaluated in the present study. In addition, sensitivity to the assessment of sensory characteristics varies between trained and untrained consumers. For example, in a study conducted with samples of *Salmo salar* (salmon), trained consumers defined 10 to 15 days in geosmin and MIB-free water as necessary to achieve the lowest level of off-flavour causing molecules in fish meat⁽²⁰⁾.

Table 2. Sensory analysis of fillets from *Prochilodus lineatus* after different depuration periods

Parameters	Depuration (hours)			
	0	48	72	96
Appearance	4.89 ± 1.39 ^a	4.84 ± 1.52 ^a	4.97 ± 1.49 ^a	4.86 ± 1.40 ^a
Color	4.90 ± 1.26 ^a	5.03 ± 1.32 ^a	5.01 ± 1.37 ^a	4.97 ± 1.27 ^a
Texture	5.27 ± 1.31 ^a	5.45 ± 0.97 ^a	5.52 ± 1.19 ^a	5.22 ± 1.25 ^a
Flavor	5.03 ± 1.35 ^a	5.42 ± 1.27 ^a	5.38 ± 1.33 ^a	5.18 ± 1.27 ^a
Overall acceptance	4.97 ± 1.42 ^a	5.25 ± 1.18 ^a	5.41 ± 1.16 ^a	5.12 ± 1.18 ^a

Results are expressed as mean ± standard deviation (n = 4). Different letters in the same row indicate significant differences between treatments by Tukey's test (p <0.05).

The pH values are shown in Figure 1A. Higher values were observed in the fillet sample from fish submitted to 48 h of depuration compared to the non-depurated, since the initial period. The same trend was found in fillets from fish submitted to 72 h of depuration, after four months of storage. The grumatã fillet depurated for 96 h showed similar pH values to those non-depurated throughout all the analysis periods.

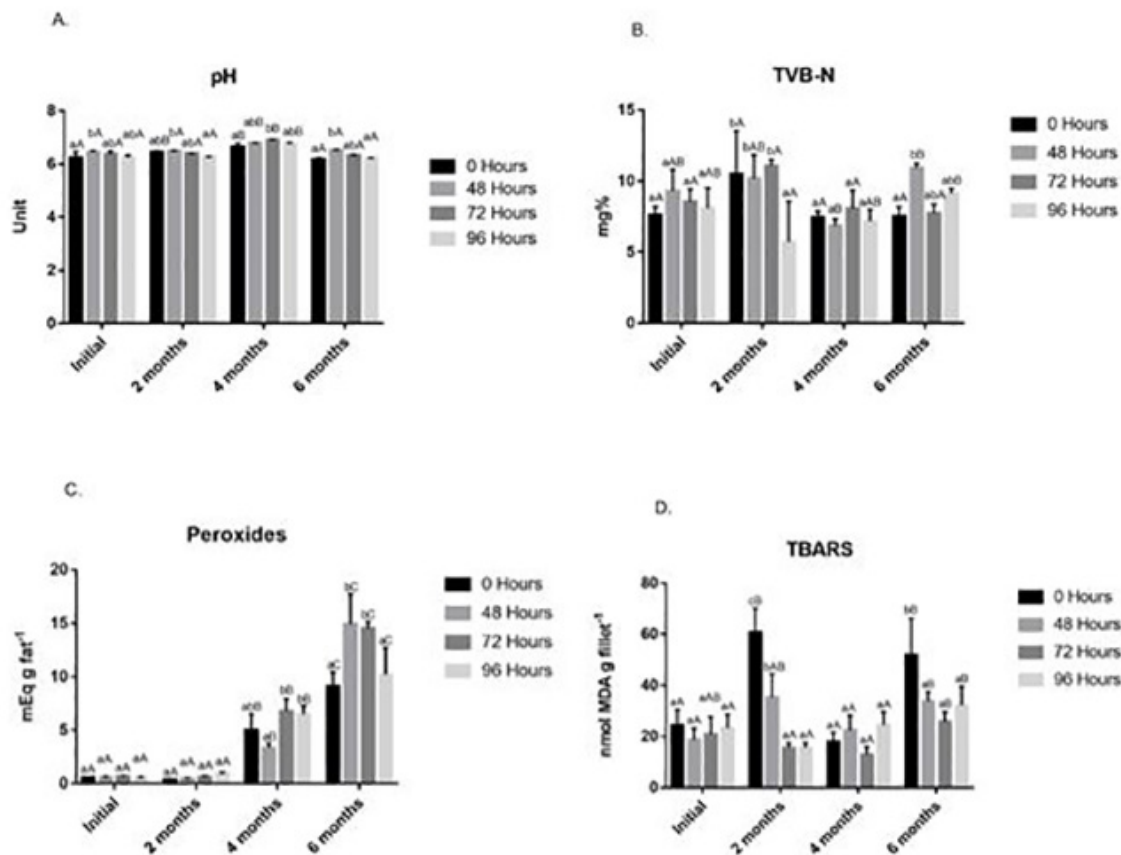


Figure 1. (A) pH; (B) Total Volatile Basic Nitrogen; (C) Peroxide and (D) TBARS values in *Prochilodus lineatus* fillet after different depuration periods. Results are expressed as mean \pm standard deviation (n = 4). Lower case letters indicate significant differences between treatments within the time period (in the month) by Tukey's test (p <0.05). Upper case letters indicate significant differences over the months within the same treatment by Tukey's test (p <0.05).

The pH and TVB-N values are the main chemical parameters used to determine the degree of freshness of fish⁽²¹⁾. Lower pH values are reached after the rigour mortis stage as a result of the anaerobic breakdown of glycogen to lactic acid, and contribute to delayed fish deterioration⁽¹⁾. In fish kept in refrigerated or frozen storage, the pH increases due to the processes of autolytic, bacterial and oxidative deterioration on proteins, fats, vitamins and minerals, which generate alkaline molecules and change the characteristics and the nutritional value of fish^(1, 22). In the present study, the pH values increased for all depuration periods during storage. However, the increase was most expressive in fillets from fish depurated for 48 h. Such degradation processes of amino compounds and other molecules can occur more intensely in this treatment which may be related to the higher protein content in the fillets. According to Brazilian law, the pH values for fish meat must be lower than 7.0 for consideration as a fresh product⁽²³⁾. Furthermore, even with the increase of pH during frozen storage, the grumatã fillets

were within the limits established by the law in all treatments.

After six months of frozen storage, the pH value of grumatã fillets reached values close to the initial ones for all depuration periods evaluated (0, 48, 72 and 96 h). A range of values was also observed in another study conducted with tilapia fish samples in frozen storage, which may be associated with the chemical changes that occur in the meat during storage (dissolution of CO₂ in fish muscle or production of volatile nitrogen bases)⁽²⁴⁾. Thus, among the depuration periods, 96 h of depuration seems to provide better pH results to contribute to the freshness of the meat, with no differences between the non-depurated, which are always lower during frozen storage. For more accurate assessment regarding the fish meat quality, the pH must be considered together with other parameters.

In relation to the TVB-N (Figure 1B), in the initial period and at four months of storage, no differences were found between different depuration periods. After two months of storage, a reduced value was observed in fillets from fish depurated for 96 h compared to other periods. After six months, the fillets from fish depurated for 48 h showed a higher value than non-depurated, with no significant differences between other depuration periods.

TVB-N are produced by enzymes endogenous to meat and of bacterial origin in fish, and act mainly on peptides, free amino acids, trimethylamine oxide, creatine, constituents of non-protein nitrogen fraction of meat, as well as proteins⁽⁹⁾. The main changes caused in nitrogen compounds are the reduction of trimethylamine oxide to trimethylamine, decarboxylation of amino acids resulting in biogenic amines (histamine, putrescine, cadaverine and others) and breakdown of urea releasing ammonia^(1, 15). The TVB-N values correspond to the volatile molecules: ammonia, trimethylamine and dimethylamine resulting from fish deterioration⁽⁹⁾. Fish with an excellent state of freshness must have 5–10 mg% nitrogen in the TVB-N form. However, Brazilian law establishes values up to 30 mg% as acceptable for consideration as a fresh product⁽²³⁾. Thus, the grumatã fillets from all depuration periods and storage conditions were within the limits established by law and could be considered as being in a good state of freshness. Additionally, this result suggests that the fillets could be stored for a longer time. The initial TVB-N content in the fish is influenced by species of fish and handling applied before and after slaughtering, such as technological processes for meat conservation⁽⁹⁾. In this way, specimens of pirarucu (*Arapaima gigas*), slaughtered and stored whole on ice, had 6.65 mg% of TVB-N at the initial period of storage and 18.76 mg% after 36 days⁽¹⁹⁾. Tilapia fillets in frozen storage showed an initial value of 12.6 mg% and at 150 days increased to 21.9 mg% of TVB-N, which indicates a relationship between these molecules, the species and all management applied pre and post-slaughter⁽²²⁾.

For the amount of peroxides (Figure 1C), no significant changes were found between depuration periods and low values were observed until two months of storage. In the fourth month, fillets from fish depurated for 48 h had a lower peroxide value compared to those depurated for 72 and 96 h. In the sixth month, higher values were found in fillets from fish submitted to 48 and 72 h of depuration, with differences between the non-depurated and those depurated for 96 h.

Fish is very susceptible to lipid oxidation due to the high level of fats and/or unsaturated fatty acids in tissues, which are subject to the action of oxidising agents, such as oxygen, metals and peroxides with generation of highly reactive substances (free radicals) and fatty acids. Consequently, its nutritional value can be reduced due to the oxidative process⁽²⁵⁾. These processes also change muscle proteins and sensory properties, such as colour, aroma, flavour and meat texture⁽¹⁾. Hydroperoxide, measured as the peroxide value, is the primary product of lipid oxidation in meat. Hydroperoxides are unstable compounds and their breakdown generates volatile molecules, such as aldehydes, ketones and alcohols that change, among other characteristics, the aroma of meat. These volatile molecules represent the second stage in the process of lipid oxidation⁽²⁶⁾. Thus, in the present study, the formation of hydroperoxides in fillets occurred more intensely after four months and was intensified after six months of storage. The fat content and the presence of pro-oxidants may have contributed to this process, which was observed in all treatments. In a study with tilapia fillets (*O. niloticus* x *T. mosambicus*), Karami et al.⁽²²⁾ found gradual increases in peroxide values after 150 days of frozen storage and showed that oxidative mechanisms occur even during frozen storage. However, in rainbow trout fillets (*Oncorhynchus mykiss*) stored in ice, during the first days of storage, higher peroxide values were observed, which indicates that lipid oxidation occurs more rapidly in this condition⁽²⁶⁾.

Data from TBARS values are shown in Figure 1D. In the initial period, TBARS values in the grumatã fillets ranged from 18.7 to 24.4 nmol MDA g⁻¹, with no significant differences between the depuration periods. After two months of storage, increased values were observed in non-depurated fish and those depurated for 48 h and lower values were found in fish depurated for 72 and 96 h. After four months in frozen storage, no significant differences were found for any of the depuration periods and the values were similar to those reported in the initial period. On the other hand, in the sixth month of storage, the grumatã fillets showed higher TBARS content in non-depurated fish compared to depurated. In relation to the initial storage period, frozen storage up to six months led to the formation of more volatile substances in fillets from non-depurated fish, with no significant differences in TBARS values for other depuration periods (48, 72 and 96 h).

The TBARS values represent volatile molecules that are formed in the second stage of lipid oxidation. The main aldehyde that reacts with thiobarbituric acid to produce colour is malonyldialdehyde, however, other unsaturated aldehydes are also reactive⁽²⁵⁾. The increase in TBARS values during frozen storage were observed for all depuration periods and could not be avoided. Similar findings were reported by Karami et al.⁽²²⁾ in a study with tilapia fillets in frozen storage for 150 days and Shi et al.⁽²⁷⁾ in Channel catfish fillets in frozen storage for up to 24 weeks. These oxidative processes that occur in lipids and proteins change the nutritional value and sensory characteristics, such as taste, odour, texture, liquid loss and colour during frozen storage⁽¹⁵⁾. In the present study, a lower increase in the formation of thiobarbituric acid reactive molecules was found in the depurated fish meat, which demonstrates a positive or delaying effect of depuration on lipid oxidation in grumatã fillets in frozen storage for up to six months.

Conclusion

Although the applied depuration periods did not change the microbiological and sensory characteristics of the grumatã fillets, the parameters related to freshness, such as pH, and the parameters related to lipid oxidation, such as TBARS content, demonstrated that the application of depuration can be considered more efficient for conservation and quality of grumatã fillets in frozen storage, mainly at 96 h of depuration.

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