Revista Ciência Agronômica, v. 52, n. 2, e20164845, 2021
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Slaughtering procedures on the flesh quality of frozen stored pacu[1]

Procedimentos de abate sobre a qualidade da carne de pacu armazenado sob congelamento

Pamela Jenny Montes Girao[2], Paulo Roberto Campagnoli de Oliveira Filho[3], Mariza Pires de Melo[4], Humber Agrelli de Andrade[3] and Elisabete Maria Macedo Viegas[2*]

ABSTRACT - Pacu (*Piaractus mesopotamicus*) is one of the most important fish farmed species in Brazil due to its hardy, fast growing, omnivorous feeding behavior, and good consumers acceptance. However, studies on the effect of different slaughtering procedures on quality of frozen pacu fillets have not been carried out. Therefore, this study aimed to evaluate the effect of electrical stunning, CO_2 narcosis, or water/ice immersion on the quality (lipid oxidation - TBARS, protein oxidation - carbonyl and thiols, protein denaturation, and instrumental color – L*, a*, and b*) of frozen (-18 °C) pacu fillets stored for up to 6 months. Electrical stunning became the fillets darker and red, besides leading to higher protein oxidation (carbonyl and thiols), and protein denaturation. CO_2 narcosis resulting in more pale flesh, with higher protein oxidation. The slaughtering by water/ice immersion was the best alternative for obtaining high quality frozen pacu fillets with lower oxidation and protein denaturation. A quality loss was observed for all pacu fillets stored at -18 °C over the months, as evidenced by the increase in lipid and protein oxidation, color changes, and higher protein denaturation. Additionaly, a maximum storage period of 3 to 4 months is recommended for frozen pacu fillets.

Key words: *Piaractus mesopotamicus*. Frozen fillets. Lipid and protein oxidation.

RESUMO - O pacu (*Piaractus mesopotamicus*) é uma das espécies de peixes cultivados no Brasil de maior importância devido a rusticidade, crescimento rápido, hábito alimentar onívoro e boa aceitação pelo consumidor. No entanto, ainda não foram realizados estudos sobre o efeito de diferentes procedimentos de abate sobre a qualidade de filés congelados de pacus. Portanto, o objetivo deste estudo foi avaliar o efeito do atordoamento elétrico, narcose por CO_2 ou imersão em água e gelo sobre a qualidade da carne (oxidação lipídica – TBARS, oxidação das proteínas – carbonilas e tiois, desnaturação das proteínas e cor instrumental – L*, a* e b*) de filés de pacus armazenados congelados (-18 °C) por até 6 meses. O atordoamento elétrico tornou os filés mais escuros e vermelhos, além de causar maior oxidação (carbonilas e tiois) e desnaturação das proteínas. A narcose por CO_2 resulta em carne mais pálida, com maior oxidação de proteínas. A morte por imersão em água e gelo foi a melhor alternativa por obter filés congelados de alta qualidade com baixa oxidação e desnaturação das proteínas. Os filés de pacus perdem a qualidade com o passar dos meses, como observado pelo aumento da oxidação lipídica e proteica, pelas mudanças na cor e aumento da desnaturação das proteínas. Além disso, recomenda-se um período máximo de armazenagem de 3 a 4 meses para filés de pacus congelados.

Palavras-chave: *Piaractus mesopotamicus*. Filés congelados. Oxidação lipídica e proteica.

DOI: 10.5935/1806-6690.20210015
Editor-in-Chief: Prof. Dr. Alek Sandro Dutra - alekdutra@ufc.br
*Author for correspondence
Received for publication on 02/03/2016; approved on 08/10/2020
[1]Projetos de pesquisa vínculados ao artigo: "Avaliação de diferentes métodos de abate sobre o bem estar e qualidade da carne de pacu (Piaractus mesopotamicus) armazenados sob congelamento" e " Avaliação de diferentes métodos de abate sobre o bem-estar e qualidade da carne de pacu (Piaractus mesopotamicus), pintado (Pseudoplatystoma corruscans) e tilápia do Nilo (Oreochromis niloticus). (CNPq e CAPES)
[2]Department of Animal Science, Faculty of Animal Science and Food Engineering/FZEA, University of São Paulo, Pirassununga-SP, Brazil, pamjmontesgirao@hotmail.com, (ORCID ID 0000-000), emviegas@usp.br (ORCID ID 0000-0002-1970-2198)
[3]Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco/UFRPE, Recife-PE, Brazil, paulocoliveira79@hotmail.com (ORCID ID 0000-0001-9060-4323), humber.andrade@gmail.com (ORCID ID 0000-0002-4221-8441)
[4]Department of Basic Sciences, Faculty of Animal Science and Food Engineering/FZEA, University of São Paulo, Pirassununga-SP, Brazil, mpmelo@usp.br (ORCID ID 0000-0003-0275-575X)

INTRODUCTION

Cold shock by ice water immersion is a slaughtering method widely used by fish processing industries in Brazil, and has been the subject of studies about flesh quality of fresh water (OLIVEIRA FILHO *et al*., 2015), and marine fish species (ALVAREZ *et al*., 2008). Another slaughtering procedure studied is the immersion in CO_2 saturated water (OLIVEIRA FILHO *et al*., 2015; VARGAS *et al*., 2013). This technique keeps the flesh quality, but causing rapid and violent reaction and an attempt to escape (POLI *et al*., 2005). Electrical stunning, which consists of passing electric current in water or directly through fish until complete loss of consciousness, is also an alternative for fish stunning (GASCO *et al*., 2014; MORZEL; SOHIER; VAN DE VIS, 2002; OLIVEIRA FILHO *et al.*, 2016). This technique leads to unconsciousness almost instantaneously. However, to prevent damage on flesh quality, optimization of variables is required (NORDGREEN *et al*., 2008; OLIVEIRA FILHO *et al.*, 2016).

After slaughtering, freezing has been widely used to keep longer the nutritional and sensory quality of fish. However, fish flesh becomes highly susceptible to lipid oxidation during frozen storage (ARANDA; MENDOZA; VILLEGAS, 2006). The oxidation products, mainly aldehydes, react with amino acids, inducing denaturation of myofibrillar proteins (CHAIJAN *et al*., 2007) and protein oxidation, which can damage the flesh texture. In addition, the lipid oxidation can also affect color, with a negative effect on the organoleptic properties (BARON *et al*., 2007). Thus, studies on lipid and protein oxidation, and protein denaturation are of great importance for assessing the quality of frozen fish.

Pacu (*Piaractus mesopotamicus*) is a Brazilian native species that shows great rusticity, omnivorous feeding behavior, satisfactory growth in captivity and good acceptance in the consumer market. Pacu has been studied in several areas such as biology (COSTA; MATEUS, 2009) and physiology (CUNHA BASTOS *et al*., 2007). However, studies on the effect of different slaughtering procedures on quality of frozen pacu fillets have not been carried out. Therefore, the aim of this study was to evaluate the effect of slaughtering methods on physicochemical variables of pacus fillets kept frozen at -18 °C for up to 6 months.

MATERIAL AND METHODS

One-hundred and fifty pacu (mean ± SD = 580.1 ± 113.2 g) were purchased from a commercial fish farm near the laboratory, transported alive, and housed in three 5,000-liter capacity masonry tanks with the following water quality parameters: dissolved oxygen of 6.1 ± 0.1 mg L^{-1}, temperature of 22.4 ± 0.2 °C, and pH 6.1 ± 0.4. Fish were fasted for 48 hours prior to the experiments.

The three slaughtering procedures were performed on the same day. The water volume of all tanks was reduced by half and fish were quickly captured and transferred to three plastic boxes (120 L), one for each slaughtering procedure. All 50 fish per treatment were slaughtered at the same time.

For electrical stunning (ES), water with salinity was adjusted with sodium chloride to contain approximately 700 µS cm^{-1} conductivity according to Lambooij *et al*. (2008). The natural water quality parameters were: dissolved oxygen 6.0 mg L^{-1}, temperature of 23 °C and pH 6.3. For the formation of the electric field, two aluminum plates (65 cm long x 35 cm wide) were placed near the box walls with a distance of 49 cm between them. In each plate, an electrode was attached to a machine specifically designed for this purpose, capable to perform the electrical discharge from 0 to 220 V. Fish were exposed to alternating electric current (AC) of 50 Hz, 200 V for 180 seconds according to Oliveira Filho *et al*. (2016).

For CO_2 narcosis (CN), pacu were transferred to plastic tanks (120 L) containing water at room temperature, and CO_2 gas was immediately injected (2 kgf cm^{-2}) into the water until total apparent stunning of animals, which occurred 30 min after CO_2 injection (OLIVEIRA FILHO *et al*., 2015). The variables related to water quality before and after CO_2 injection were: dissolved oxygen before and after stunning = 6.4 mg L^{-1}, temperature before = 21.3 °C; temperature after = 21.1 °C; pH before = 6.6; and pH after stunning = 4.5.

For slaughtering by ice/water immersion (IW) (1:1), fish were placed simultaneously in plastic box containing a mixture of 60 L water and 60 L of ice flakes according to Oliveira Filho *et al*. (2015). The pacu took about 20 minutes to lose the apparent sensitivity. Water quality parameters at the beginning and at the end of the stunning procedure were: dissolved oxygen before = 19.9 mg L^{-1} and after stunning = 18.7 mg L^{-1}; temperature before = 1.3 °C and temperature after stunning = 1.5 °C; pH before = 6.4; and pH after stunning = 6.5.

For all treatments, the apparent stunning was confirmed by loss of fish balance, stop of opercular movements (breathing), absence of activity in response to lateral-line stimulation, and no vestibulo-ocular reflex (VOR), according to Oliveira Filho *et al.* (2016). After this procedure fish were then slaughtered with manual cutting of the four gill arches, and sangria was performed in running water for about 3 min. Fish were washed with chlorinated water (5 ppm of free chlorine), and filleted pre-rigor mortis. Subsequently, the fillets underwent

ultra-rapid freezing in a freezer shelf for about 40 minutes until the internal temperature reached -30 °C, packed in individual polyethylene bags, with hermetic sealing and stored frozen (-18 ºC) in the absence of light until the analyses. The chemical composition was determined (moisture = 72 ± 1%, protein = 18 ± 1%, lipids = 8 ± 1%, and ash = 2 ± 1%) according to the methods described in Association of Official Analytical Chemists (2012). Before each analysis, fish fillets were thawed at 7 °C for about 24 hours. Laboratory tests were performed in three thawed fillets per treatment on the first day after freezing (time 0) and after 2, 4 and 6 months of storage at -18 °C.

The thiobarbituric acid reactive substances (TBARS) method was used for analysis of lipid oxidation. The fillets were pre-homogenized in a food processor according to Vyncke (1970). TBARS were calculated (absorbance x = 48.946 [TMP] + 0.0028; $R^2 = 0.99$) using tetramethoxypropane (TMP) as standard precursor, and the results were expressed as malonaldehyde mg kg^{-1} sample.

The protein oxidation was determined by measuring the carbonyl groups and free thiols. The analysis of carbonyl groups was performed according to Mercier, Gatellier and Renerre (2004) with modifications. For that, 1 g of white muscle from different storage periods was homogenized with 20 mL of extraction buffer (100 mM sodium phosphate, pH 7.0) for 60 seconds, and then filtered through qualitative filter paper (Whatman ≠1). The total protein content was determined at 280 nm according to Bradford method (BRADFORD, 1976), using a standard curve of albumin. The carbonyl content was assessed with dinitrophenylhydrazine (DNPH) measured at 370 nm in a UV/Visible spectrophotometer using the molar extinction coefficient of DNPH (22,000 M^{-1} cm^{-1}). The results were expressed as nmol mg^{-1}.

The free protein thiols groups in white muscle were measured using 5,5-Dithiobis (2-nitrobenzoic acid) DTNB, according to Ellman (1959). The method consisted of two steps: 1) Extraction of myofibrillar and sarcoplasmic proteins using 5% SDS in 0.1M Tris-HCl buffer (pH 8.0); 2) Determination of thiols by derivation with DTNB. For that, 1 g of sample was homogenized with extraction buffer (0.1 M Tris-HCl buffer, pH 8.0) and analyzed the absorbance of DTNB at 412 nm in UV/Visible spectrophotometer. The thiol content was calculated as nmol of thiol per mg protein. The total protein content was determined according to Bradford method (BRADFORD, 1976), using a standard curve of albumin.

The denaturation of myofibrillar proteins of the fillets was evaluated by differential scanning calorimetry (DSC). Analyses of denaturation temperature and enthalpy for actin and myosin were performed in the thawed fillets at 7 °C operating with a gas flow of 45 mL N_2 min^{-1}, heating rate of 10 °C min^{-1}, ranging from 0 to 100 °C, with an empty reference pan according to Oliveira Filho *et al*. (2011).

The instrumental color was determined on fillets. On region above the lateral line at three points near the skull, using a portable colorimeter (Miniscan XE, Hunterlab, Reston, USA), previously calibrated with black and white pattern before each analysis, using D65 light source, observation angle of 10º and opening of measuring cell of 30 mm. Color was expressed using the CIELab color standard system - "Commission Internationale de L'Eclairage": L^* (lightness), a^* (intensity of red-green color) and b^* (intensity of yellow-blue color).

This work was approved by the Committee on Ethics in Research of the University of São Paulo, Faculty of Animal Science and Food Engineering – USP/FZEA (protocol no. 13.1.2333.74.5).

The experimental design was completely randomized with three treatments (water/ice slurry, CO_2 narcosis, and electric shock) at time intervals (on the first day after freezing, time 0, 2, 4 and 6 months). The response variables of interest in the statistical analysis are lipid oxidation (TBARS), protein oxidation (carbonyl and thiols), denaturation temperature and enthalpy for actin and myosin, and instrumental color. The explanatory variables were the three slaughtering procedures and the frozen storage period, which was considered in the original scale and the square to accommodate nonlinearities in temporal variations of the variable responses. The first order interactions between the explanatory variables were also considered.

For the analysis, generalized linear models were used in the form $g(E(Y)) = X\beta$, where Y is the dependent variable, X is the design matrix of the explanatory variables, and β is the vector of parameters to be calculated, representing the effects of the explanatory variables. The letters g and E correspond to the link function and expectation. Identity was used as a coupling function and it is assumed that Y follows a normal distribution. The model corresponds to an analysis of covariance (ANCOVA). Diagnostic of residuals, with comparisons between the adjusted and observed values, scale and quantile graphics were used to assess the validity of the proposed model.

To select the variables (treatment, time, time 2, and their interactions) relevant to explain the variations in responses of interest (e. g. carbonyl), the information criterion was used using backward selection. The Section *Results and Discussion* only presents the results for the model with the variables selected as previously described, and the generalized linear models with significant differences are highlighted ($\alpha = 0.05$). All analyses were performed using the R software.

RESULT AND DISCUSSION

Although no significant differences were observed in the lipid oxidation (TBARS values) of the frozen pacu fillets ($P > 0.05$) for all slaughtering procedures, an effect ($P < 0.05$) of time was observed, with a non-linear increase and a trend towards stabilization in the final period of frozen storage (Figure 1). Curves were not observed, since no significant differences were found for all treatments. Stabilization at the end of the storage period may have occurred, once the oxidation compounds may have transformed into other compounds over time, and the method of analysis did not detect the formation of malonaldehyde. After 6 months of storage, the pacu fillets showed TBARS values of approximately 1.5 mg malonaldehyde kg^{-1}. High TBARS values during storage may be due to the high lipids content in the pacu fillets (8 ± 1%). Brazilian law has not established an upper limit of TBARS levels for lipid oxidation in fish. However, the formation of toxic compounds such as aldehydes, ketones, alcohol, acids, and hydrocarbons may occur in food in advanced stages of oxidation (CHAIJAN *et al*., 2007). In addition, the lipid oxidation can cause the oxidation of pigments, affecting the sensory attributes including color (ARANDA; MENDOZA; VILLEGAS, 2006; BARON *et al*., 2007), rancid flavor, and protein oxidation, causing protein denaturation (ESTÉVEZ *et al*., 2011; LUND *et al*., 2011).

The formation of carbonyls is one of the most prominent changes in oxidized fish proteins (ESTÉVEZ *et al*., 2011). In this study, differences ($P < 0.05$) were observed in the initial (time 0 - on the first day after freezing) carbonyls values in the pacu fillets subjected by ES (higher) and IW (lower) (Figure 2a). The protein oxidation adversely affects the solubility and the functional properties, thus reducing gelling, emulsifying, and water holding capacities (BARON *et al*., 2007; LUND *et al*., 2011), and protein digestibility with destruction of amino acids, besides changes in taste and color (ESTÉVEZ *et al*., 2011; LUND *et al*., 2011).

A decrease ($P < 0.05$) in carbonyl values was observed during frozen storage of pacu fillets subjected by ES, with few variations in fish slaughtered by IW or CN (Figure 2a). For these two cases, the storage period did not greatly affect in the formation of carbonyl groups, which ranged from approximately 3 to 5 nmol mg^{-1} protein. In trout (*Oncorhynchus mykiss*) stored frozen for up to 13 months, in contrast to the present study, there was a considerable increase in the carbonyl groups from 2 to 7.7 nmol mg^{-1} protein (BARON *et al*., 2007). According to Estévez *et al*. (2011), the carbonylation of meat proteins during frozen storage can be affected by several factors such as lipid oxidation, type of animal, storage temperature, packaging conditions, and previous industrial operations.

The thiol group of cysteine is highly susceptible to oxidation in the presence of hydrogen peroxide accumulated in fish cells after death. The increased protein oxidation leads to the loss of thiol groups, and the rate of these reactions will depend on muscle type, experimental conditions, and type and time of storage (LUND *et al*., 2011). The thiols values at time 0 were higher in pacu fillets slaughtered by IW, when compared with those slaughtered by CN or ES (Figure 2b). This is an indication that slaughtering by IW led to less oxidation of myofibrillar proteins in pacu after death. However, during storage a reduction in thiols values was observed in fish subjected to slaughtering by IW, while values with slight upward trend were observed in fish subjected to the other two slaughtering procedures (Figure 2b). Since the analysis of protein oxidation is a relatively new issue, this variation is still difficult to explain. The issue is controversial because in a study on refrigerated horse mackerel (*Trachurus trachurus*), Baron *et al*. (2007), also observed an increase in thiols values, while another study found a decrease of up to 4% of the initial concentration of thiols during storage of the same species (LUND *et al*., 2011). Therefore, further studies are needed to elucidate the variation of thiols in fish flesh throughout the frozen storage.

Protein denaturation is an order-disorder transition that does not change the primary amino acid sequence, but modifies the protein conformation (OLIVEIRA FILHO *et al*., 2011). The protein denaturation may occur due to external agents (storage temperature, air pressure, mechanical strength, and stress pre slaughter) and internal agents (chemical composition, pH, living habits, and

Figure 1 - TBARS values of pacu fillets submitted by electrical stunning (ES), CO_2 narcosis (CN) or iced water (IW) and stored frozen (-18 °C) for up to 6 months. The symbols represent the observed values and the line model fit

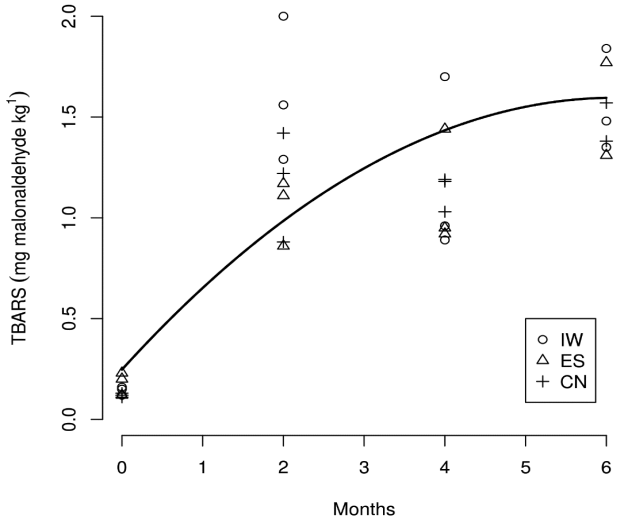
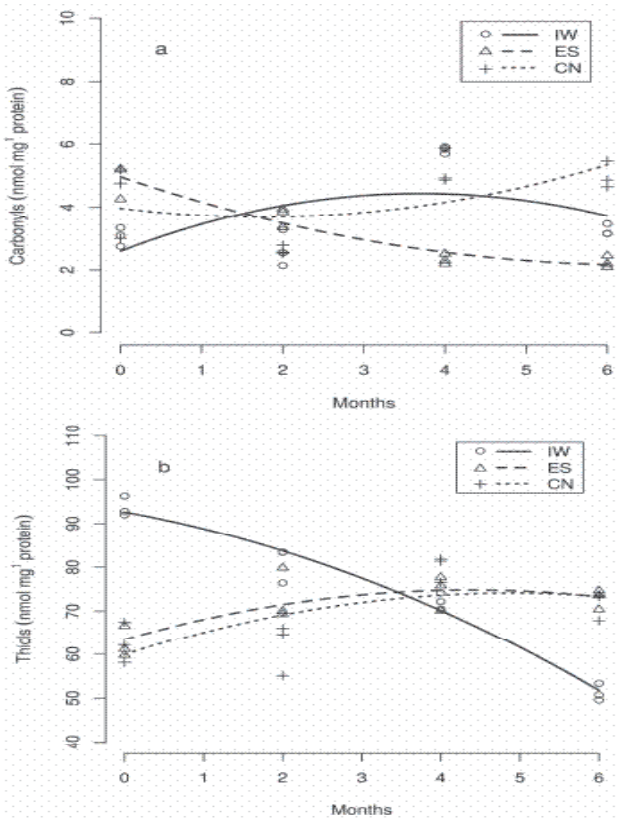


Figure 2 - Protein oxidation (carbonyls - a and thiols - b) of pacu fillets submitted by electrical stunning (ES), CO_2 narcosis (CN) or iced water (IW) and stored frozen (-18 °C) for up to 6 months. The symbols represent the observed values and the line model fit



nutritional status) (MATOS *et al*., 2010; OLIVEIRA FILHO *et al*., 2011). The denaturation of fish proteins during frozen storage can lead to changes in color and texture, in addition to affecting gel formation in processed products such as surimi (JENSEN; JORGENSEN; NIELSEN, 2003; SAEED; HOWELL, 2004).

Myosin corresponds to the thick filament of myofibrils and undergoes denaturation in fish meat in the temperature range of 30 to 60 °C (HERRERA; PASTORIZA; SAMPEDRO, 2001), and is highly influenced by the animals habits (PAREDI *et al*., 1994). In the present study, no changes were observed in the denaturation temperature of myosin ($P > 0.05$) for all slaughtering procedures and during frozen storage, with a mean temperature of 53.3 ± 1.0 °C. This result is similar to that observed for Nile tilapia (*Oreochromis niloticus*) (54 °C) (OLIVEIRA FILHO *et al*., 2011). The denaturation temperature and enthalpy of myosin at time 0 was lower ($P < 0.05$) in the pacus subjected to ES when compared to those subjected to CN and IW, which did not differ ($P > 0.05$) among them (Figure 3a), suggesting that the proteins of the ES treatment were more denatured than those of CN and IW treatments. This fact shows that the ES was not adequate to stun pacu, once the lower denaturation enthalpy means that a larger portion of myosin has already denatured before the analysis. Thus, it may be hypothesized that the electric current for pacu stunning was inadequate, which negatively affected flesh quality. During storage, the denaturation enthalpy of myosin in pacu fillets subjected to CN or IW tended to increase ($P < 0.05$), whereas the values of the fillets subjected to ES remained practically constant over 6 months (Figure 3a). This fact is interesting because it shows that the myosin from fish subjected to ES may be already denatured after death, while in the other slaughtering procedures the myosin denaturation occurred gradually. According to Jensen, Jorgensen and Nielsen (2003), the protein denaturation of frozen fish is a common phenomenon and occurs by several factors such as the action of autolytic enzymes and oxidation of lipids and proteins.

Actin corresponds to the thin filament of myofibril and undergoes denaturation from 65 to 85 °C (PAREDI *et al*., 1994). No changes ($P > 0.05$) were observed in the denaturation temperature of actin for all slaughtering procedures and during frozen storage, with a mean temperature of 77.4 ± 0.3 °C. This result is similar to that observed for seabream (*Sparus aurata*) in the pre-slaughter stress, with values close to 74 °C (MATOS *et al*., 2010). The denaturation temperature of actin in the pacu of the present study was higher than that observed for Nile tilapia (*Oreochromis niloticus*) (73.9 °C) (OLIVEIRA FILHO *et al*., 2011) and cholga mussel (*Aulacomya ater ater*) (72 °C) (PAREDI *et al*., 1994).

The denaturation enthalpy of actin at the beginning of the process (time 0) was lower ($P < 0.05$) in pacu subjected to ES (Figure 3b), demonstrating that this treatment has led to a greater denaturation of actin. A smaller denaturation enthalpy of actin was also observed in seabream (*Sparus aurata*) subjected to a higher pre-slaughter stress (MATOS *et al*., 2010). During frozen storage, the denaturation enthalpy of actin in fish slaughtered by CN or IW tended to decrease ($P < 0.05$), whereas the values of the fillets subjected to ES remained practically constant over 6 months of frozen storage (Figure 3b).

Lightness (L^*) was significantly lower ($P < 0.05$) in the pacu fillets subjected to ES when compared to CN and IW, which did not differ ($P > 0.05$) between them (Figure 4a). Some authors observed darkening of fish fillets subjected to electrical stunning when compared to other slaughtering procedures (LAMBOOIJ *et al*., 2008; VARGAS *et al*., 2013). During frozen storage of pacu fillets, no changes were observed for the L^* values ($P > 0.05$) in all treatments. This is a positive factor because over time the frozen fillets tend to undergo browning due to

protein denaturation (GARTHWAITE, 1997). Thus, the maintenance of lightness evidences that both rapid freezing and frozen storage of the fillets were performed properly.

A difference ($P < 0.05$) in redness (a^*) was observed in the pacu fillets subjected to CN (less red) and ES (more red) at time 0 (Figure 4b). Other studies have also found higher a^* values in fish subjected to ES in relation to other slaughtering methods (LAMBOOIJ *et al*., 2008; VARGAS *et al*., 2013). Bleeding in fish fillets lead to lower sensory quality and can be vehicles of microbiological contamination besides reducing shelf life, once the blood is a catalyst for lipid oxidation (SÁNCHEZ-ALONSO; BORDERÍAS, 2008). During frozen storage, the red color of pacu fillets showed linear behavior and a positive slope, probably due to oxidation of pigments during storage, making the fillets reddish.

No differences ($P > 0.05$) were observed in the yellow color intensity (b^* values) for all slaughtering methods (Figure 4c). In addition, differences in b^* values were not observed in several species, including Nile tilapia (*Oreochromis niloticus*) slaughtred by CO_2 narcosis or water/ice immersion (OLIVEIRA FILHO *et al*., 2015), turbot (*Psetta maxima*) slaughtered by bleeding, electric shock or cranial percussion (MORZEL; SOHIER; VAN DE

Figure 3 - Differential scanning calorimetry (DSC) of myosin denaturation enthalpy - a, actin denaturation enthalpy - b of pacu fillets submitted by electrical stunning (ES), CO_2 narcosis (CN) or iced water (IW) and stored frozen (-18 °C) for up to 6 months. The symbols represent the observed values and the line model fit

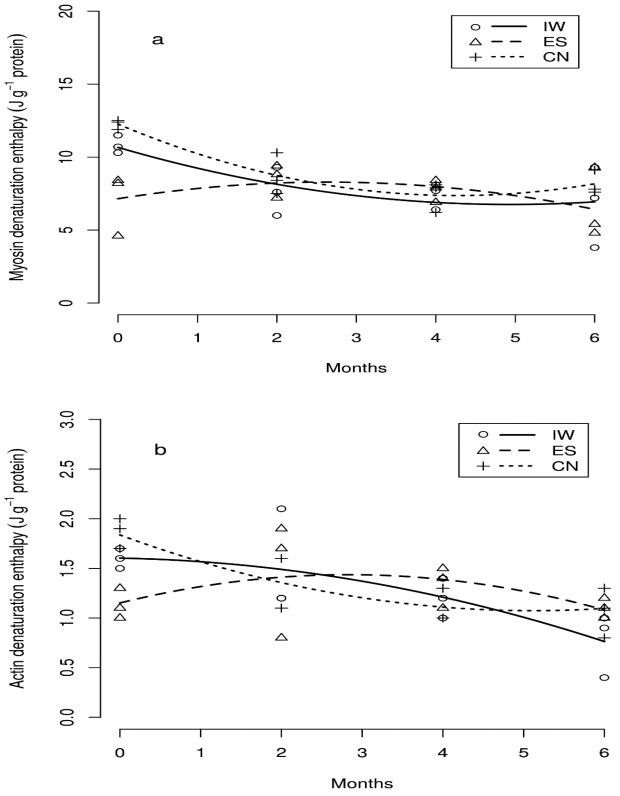
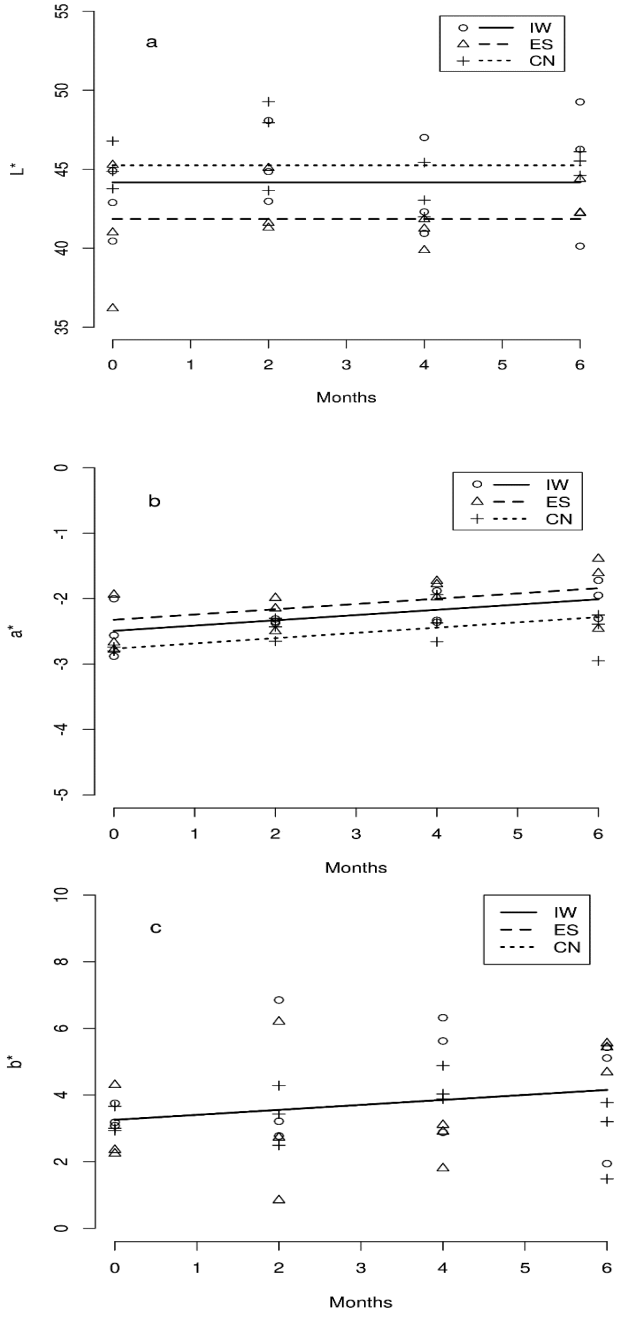


Figure 4 - Color L^* (lightness) - a, a^* (intensity of red-green) - b, b^* (intensity of yellow-blue) - c of pacu fillets submitted by electrical stunning (ES), CO_2 narcosis (CN) or iced water (IW) and stored frozen (-18 °C) for up to 6 months. The symbols represent the observed values and the line model fit



VIS, 2002), and matrinxãs (*Brycon cephalus*) slaughtered by electric shock, water/ice immersion, or CO_2 narcosis (VARGAS *et al*., 2013). During 6 months of storage, a linear increase ($P < 0.05$) in b^* values was observed, with no significant difference ($P > 0.05$) among the treatments (Figure 4c). Similar results were found in matrinxãs (*Brycon cephalus*) subjected to different slaughtering procedures and stored under refrigeration (VARGAS *et al*., 2013). The increase in yellow color intensity can be due to the myoglobin degradation during the deterioration process. Thus, newly dead fish have bluish-white meat (lower b^* value), which tends to be yellow (higher b^* value) throughout the storage (GARTHWAITE, 1997).

CONCLUSIONS

1. The slaughtering procedure by water/ice immersion was the best alternative for obtaining high quality frozen fillets with lower oxidation and protein denaturation;
2. A quality loss was observed for all pacu fillets stored at -18 °C over the months, evidenced by increase in lipid and protein oxidation, color changes, and higher protein denaturation;
3. A maximum storage period of 3 to 4 months is recommended for frozen pacu fillets.

ACKNOWLEDGEMENTS

The authors thank "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Programa Nacional de Pós-Doutorado" and "Conselho Nacional de Desenvolvimento Científico e Tecnológico/Pós-Doutorado Júnior", for granting postdoctoral scholarships for the first and second author of this article, and also the staff and students of the Laboratory of Aquaculture, Biological Chemistry and Food Technology – Faculty of Animal Science and Food Engineering, University of São Paulo - Pirassununga / SP / Brazil for the assistance in implementing this project.

REFERENCES

ALVAREZ, A. *et al.* The influence of starvation time prior to slaughter on the quality of commercial-sized gilthead sea bream (*Sparus aurata*) during ice storage. **Aquaculture**, v. 284, p. 106-114, 2008.

ARANDA, M.; MENDOZA, N.; VILLEGAS, R. Lipid damage during frozen storage of whole jack mackerel (*Trachurus Symmetricus Murphyi*). **Journal of Food Lipids**, v. 13, p. 155-166, 2006.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis**. 19 th ed. Washington, D.C.: AOAC, 2012.

BARON, C. B. *et al.* Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*). **Journal of Agricultural and Food Chemistry**, v. 55, p. 8118-8125, 2007.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. **Analytical Biochemistry**, v. 72, p. 248-254, 1976.

CHAIJAN, M. *et al*. The effect of freezing and aldehydes on the interaction between fish myoglobin and myofibrillar proteins. **Journal of Agricultural and Food Chemistry**, v. 55, p. 4562-4568, 2007.

COSTA, R. M. R.; MATEUS, L. A. F. Reproductive biology of pacu, *Piaractus mesopotamicus* (Holmberg, 1887) (Teleostei: Characidae) in the Cuiabá River Basin, Mato Grosso, Brazil. **Neotropical Ichthyology**, v. 7, p. 447-458, 2009.

CUNHA BASTOS, V. L. F. *et al*. Cytosolic glutathione peroxidase from liver of pacu (*Piaractus mesopotamicus*), a hypoxia-tolerant fish of the Pantanal. **Biochimie**, v. 89, p. 1332-1342, 2007.

ELLMAN, G. L. Tissue sulfhydryl groups. **Archives of Biochemistry and Biophysics**, v. 82, p. 70-77, 1959.

ESTÉVEZ, J. *et al.* Inhibition with spontaneous reactivation of carbonyl estereases by organophosphorus compounds: paraoxon as a model. **Chemical Research in Toxicology**, v. 24, p. 135-143, 2011.

GARTHWAITE, G. A. Chilling and freezing of fish. *In*: HALL, G. M. **Fish processing technology**. 2. ed. London: Chapman and Hall, 1997. p. 93-118.

GASCO, L. *et al.* Effects of different slaughtering methods on rigor mortis development and flesh quality of tench (*Tinca tinca*). **Journal of Applied Ichthyology**, v. 30, p. 58-63, 2014.

HERRERA, J.; PASTORIZA, L.; SAMPEDRO, G. A DSC study on the effects of various maltodextrins and sucrose on protein changes in frozen-stored minced blue whiting muscle. **Journal of the Science of Food and Agriculture**, v. 81, p. 337-384, 2001.

JENSEN, K. N.; JORGENSEN, B. M.; NIELSEN, J. Effect of storage conditions on differential scanning calorimetry profiles form thawed cod muscle. **Lebensmittel-Wissenschaft & Technologie**, v. 36, p. 807-812, 2003.

LAMBOOIJ, E. *et al*. A humane protocol for electro-stunning and killing of Nile tilapia in fresh water. **Aquaculture**, v. 275, p. 88-95, 2008.

LUND, M. N. *et al.* Protein oxidation in muscle foods: a review. **Molecular Nutrition and Food Research**, v. 55, p. 83-95, 2011.

MATOS, E. *et al.* Effect of harvesting stress and slaughter conditions on selected flesh quality criteria of gilthead sea bream (*Sparus aurata*). **Aquaculture**, v. 305, p. 66-72, 2010.

MERCIER, Y.; GATELLIER, P.; RENERRE, M. Lipid and protein oxidation in vitro, and antioxidant potential in meat

from Charolais cows finished on pasture or mixed diet. **Meat Science**, v. 66, p. 467-473, 2004.

MORZEL, M.; SOHIER, D.; VAN DE VIS, H. Evaluation of slaughtering methods for turbot with respect to animal welfare and flesh quality. **Journal of the Science of Food and Agriculture**, v. 82, p. 19-28, 2002.

NORDGREEN, A. H. *et al.* Effect of various electric field strengths and current durations on stunning and spinal injuries of Atlantic herring. **Journal of Aquatic Animal Health**, v. 20, p. 110-115, 2008.

OLIVEIRA FILHO, P. R. C. *et al.* How stunning methods affect the quality of Nile tilapia meat. **CyTA – Journal of Food**, v. 13, p. 56-62, 2015.

OLIVEIRA FILHO, P. R. C. *et al.* Impact of electrical stunning on fish behavior and meat quality of pacu (*Piaractus mesopotamicus*). **Acta Scientiarum. Technology**, v. 38, n. 1, p. 81-88, 2016.

OLIVEIRA FILHO, P. R. C. *et al.* Protein denaturation during the processing of sausage prepared with Nile tilapia fillet and minced fish. **Italian Journal of Food Science**, v. 23, p. 194-201, 2011.

PAREDI, M. E. *et al.* Thermal denaturation of *Aulacomya ater ater* (Molina) myofibrillar proteins: a differential scanning calorimetric study. **Journal of Agricultural and Food Chemistry**, v. 42, p. 873-877, 1994.

POLI, B. M. *et al.* Fish welfare and quality as affected by pre-slaughter and slaughter management. **Aquaculture International**, v. 13, p. 29-49, 2005.

SAEED, S.; HOWELL, N. K. Rheological and differential scanning calorimetry studies on structural and textural changes in frozen Atlantic mackerel (*Scomber scombrus*). **Journal of the Science of Food and Agriculture**, v. 84, p. 1216-1222, 2004.

SÁNCHEZ-ALONSO, I.; BORDERÍAS, A. J. Technological effect of red grape antioxidant dietary fibre added to minced fish muscle. **International Journal of Food Science and Technology**, v. 43, p. 1009-1018, 2008.

VARGAS, S. C. *et al.* Evaluation of different stunning methods on aspects of animal welfare and meat quality of matrinxã (*Brycon Cephalus*). **Italian Journal of Food Science**, v. 25, p. 255-262, 2013.

VYNCKE, W. Direct determination of the thiobarbituric acid value in trichloroacetic extracts of fish as a measure of oxidative rancidity. **Fette-Seinfen Anstrichmittel**, v. 72, p. 1084-1087, 1970.