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ANIMAL PRODUCTION

Physical, chemical and sensory evaluation of meat from cobia (*rachycentron canadum*), desensitized with different voltages of electric shock, stored under refrigeration

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ABSTRACT: *Fish quality is conditioned by several factors, among them desensitization methods applied for fish managing and slaughtering. This research used electric shock at different intensities as a desensitization method for cobia (Rachycentron canadum) slaughtering and evaluated its effects over fish quality during refrigerated storage. The experiment was carried out by numbing the fish with electroshock at the intensities of 100, 150 and 200 Volts, and keeping them in refrigerated storage for 21 days. On the cobia, obtained from a commercial fish farm the following physical, chemical and sensorial characteristics as quality variables were evaluated: dielectric properties, rigor index, pH, texture, color, sensorial evaluation of freshness, ATP and degradation catabolites. The experimental design was a 6×3 factorial (6 times of analysis and 3 electroshock intensities). Significant differences (P<0.05) were observed for the analyzed variables, as a consequence of desensitization treatments and of storage time within each treatment. However, the number of variables affected by the treatment was smaller than the number of variables significantly affected by the storage time, and the interaction time×treatment was highly significant within the results reported. The best results for the parameters tested were obtained when the treatment at 150 volts intensity with the majority of analyzed variables held significant differences (P<0.05) among treatments. It was also concluded that cobia can be conserved in refrigerated storage within seven days after slaughtering using electro-narcosis as a method of desensitization.*
Key words: *stocking, freshness, welfare, aquaculture.*

Avaliação física, química e sensorial de carne de cobia (*rachycentron canadum*), desensitizada com diferentes voltagens de choque elétrico, armazenadas em refrigeração

RESUMO: *A qualidade do pescado está condicionada a diversos fatores, entre eles os métodos de insensibilização. Nesta pesquisa foi utilizado o choque elétrico em diferentes intensidades como método de insensibilização do bijupirá (Rachycentron canadum), e avaliados seus efeitos sobre a qualidade dos peixes resfriados. O experimento foi realizado insensibilizando os peixes com eletrochoque de intensidades de 100, 150 e 200 Volts, e armazenados refrigerados durante 21 dias. Os peixes utilizados foram obtidos de uma piscicultura comercial localizada no litoral norte do estado de São Paulo a cerca de 50km, por via marítima, da cidade de Ubatuba-SP. As variáveis de qualidade dos peixes avaliadas foram características físicas, químicas e sensoriais como medidas dielétricas, índice de Rigor Mortir, pH, textura, cor, avaliação sensorial do frescor, ATP e produtos da degradação. O delineamento experimental utilizado foi um fatorial 6X3, seis tempos de análise e três intensidades de choque elétrico. Foram observadas diferenças significativas (P<0,05) para a maioria das variáveis analisadas, tanto para os tratamentos em relação ao tempo, como também para a influência do tempo de armazenamento dentro de cada tratamento. No entanto, o número de variáveis com significância entre os tratamentos foi menor em relação ao número de variáveis em que houve significância para os tempos de conservação, sendo a interação tempoxtratamento a que obteve significância expressiva dentro dos resultados encontrados. Foram observados os melhores resultados para o tratamento com a intensidade em 150 volts para a maioria das variáveis analisadas em que houve diferenças significativas (p<0,05) entre os tratamentos. Concluiu-se também que o bijupirá pode ser conservado em até sete dias após abate em conservação refrigerada utilizando a eletronarcose como método de insensibilização.*
Palavras chave: *estocagem, frescor, bem estar, aquicultura.*

INTRODUCTION

Cobia (*Rachycentron canadum*) is a species with high zootechnical potential, due the high growth rate, low mortality, good feed efficiency and high market value. The positive performance obtained during the rearing of cobia led Taiwan and other countries to develop an appropriate technology for its production in aquaculture farms (BENETTI, 2008). According to the FAO (2016) statistics, world production of cobia reached 40,290 tons in 2014, a very significant value for a species for which the

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entire technological breeding package has not yet been developed.

According to ERIKSON (2012), recent research indicated that fish can feel fear and pain, and that approach like this should be used in industrial aquaculture, in search for improvement in aquaculture production quality. According to the European Food Safety Authority (EFSA, 2009), all stages of fish production are critical points that may compromise fish quality and welfare. As a consequence of the stress that animals undergo during slaughter, a series of physiological reactions occur simultaneously, modifying the blood profile regarding cortisol concentration, a chemical indicator of stress (LUPATSCH et al., 2010).

Electroshock, or eletronarcosis, has been used in tests due to its action of causing unconsciousness in less than one second (EFSA, 2009). The success of eletronarcosis use can be proven by electrocardiograms and electroencephalograms (VAN DE VIS et al., 2003). However, ROTH et al. (2009) show that eletronarcosis causes rupture of the blood vessels causing dark spots in the muscular tissue and may even cause rupture in fish vertebrae, which diminishes fish quality and its potential commercialization.

Both the stunning and the time when the animal remains numbed depend on factors such as water conductivity, fish orientation, voltage, electric current and electric field, but it is observed that after the desensitization by eletronarcosis, the use of water or ice causes death by hypoxia, even before the fish regain consciousness. The arrangement of the electrodes in relation to the fish should be observed because the touch of the caudal fin can cause the pre-shock, the flow of the electric current from the tail to the head, stimulating the escape and increasing the animal's stress level (LINES and SPENCE, 2014).

The present study aimed to evaluate electronarcosis effects on physical-chemical and sensorial quality of the cobia (*Rachycentron canadum*) meat stored under refrigeration for 21 days.

MATERIALS AND METHODS

Location and animals

The experiment was carried out in Ubatuba - SP, in the Clarimundo de Jesus base belonging to the Oceanographic Institute of University of São Paulo, and in the Aquaculture Laboratory of the Animal Science and Food Engineering Department (FZEA) of the University of São Paulo (USP), campus of Pirassununga (SP, Brazil). The specimens, weighing approximately 2.0±0.4kg, were obtained from a commercial production in the city of Ubatuba (SP, Brazil) where they were grown in net tanks and fed with fishing tailings. They were transported by sea in transportation tanks equipped with oxygen, then submitted to electric stunning by application of the following three treatments differing for current voltage applied for three minutes: 100, 150 and 200 volts in alternating current. To evaluate the effect of the different current on the quality of cobia during the storage, the fish were kept under refrigerated condition at 4ºC for 21 days was evaluated.

During the storage period, periodic samplings of the cobia were carried out to evaluate the sensorial characteristics of freshness, ATP content and rate of degradation and dielectric properties immediately after death (time 0), at 3 and 5 hours after death, and 7, 14 and 21 days after death. Muscle pH was analyzed at the times 0, 3 and 5 hours, and at 7, 14, 21 and 28 days after death, while for the color and the texture were measured at the times 7, 14 and 21 days after death.

Sensory evaluation of freshness

The sensorial evaluation was carried out in all treatments by five judges, specialists of the area, using the European Union official scheme (European Union Regulation, CEE 103/76 modified by the Regulation 2406/96). The EU scheme establishes four levels of fish freshenss (E–extra, score=3; A–fresh, score=2; B-no fresh, rancid, score=1;), Unfit-deteriorated, score=0) for all the sampling points.

Rigor index (IR)

After death, all the fish of each treatment were measured for total length and weighed using an ichthyometer and digital balance, identified with labels and submitted to initial measurement needed for calculation of *rigor* index according to the methodology described by BITO et al. (1983).

In order to analyze the evolution of *rigor* index during the post mortem period, three fish from each treatment at each time were submitted to the rigor index (IR) measurement, according to BITO et al. (1983).

$$IR=D-D0\times100/D0$$

where

IR: rigor index;

D0: Initial distance between table surface and caudal fin base;

D: Final distance between table surface and caudal fin base.

Muscle pH

It is performed with the aid of a pH meter. The sensor of the instrument was introduced in an aqueous solution obtained mixing 10g of muscle sample in 40mL of distilled water (BRASIL, 1981).

Texture

The analysis of texture was performed instrumentally in three different points (cranial, medial and caudal) of three cobia fillets for each treatment and each time point, using a pre-calibrated with standard texturometer (TA-XT2i, Stable Micro Systems), pre-set at the standard weight of 5kg, equipped with an aluminum probe (SMS P/20) with pre-test, test and post-test speed of 2.0mm/s and a platform height of 20mm. The compressive force (g) was analyzed using a 40% fillet height (BOURNE, 2002).

Color

The instrumental color measurement was performed on three left fillets of fish from each treatment at 7, 14 and 21 days of storage by a portable colorimeter (Miniscan XE, Hunterlab), previously calibrated with white and black standard before each analysis, using D65 light source, 30mm cell aperture viewing angle of 10º. The values of color were expressed using the color standard parameters of the CIELab system -, where L^* is the lightness, a^* is the redness index (varying from red to green color) and b^* is the yellowness index (varying from yellow to blue color).

Dielectric properties

The dielectric measurements of the cobia were made with the aid of the Fish Freshness Meter, applying the instrument directly in contact with the fish skin.

ATP and related catabolites

The concentrations of adenosine triphosphate (ATP) and its degradation by-products, i.e. adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) were determined by high precision liquid chromatography (HPLC) according to BURNS et al. (1985). For the mobile phase, a solution of KH_2PO_4 (0.6M) with a flow of 1.4mL min^{-1} was used in reverse phase analytical column (RP 8MPLC, dimensions: 4.6mm×10cm, particle size 10μm, embedded in a protective column, 4.6mm×3cm, particle size of 10μm). The K value was determined by the ratio between the total amount of HxR and Hx and the total amount of ATP and its degradation catabolites in muscle, according to the formula proposed by SAITO et al. (1959):

K (%) = [(HxR / ATP + ADP + AMP) + (Hx / IMP+HxR+Hx)]x100

Statistical analysis

A 6×3 factorial test involving six time points (0, 3, 5, 168, 336 and 504 hours after slaughter) and three electroshock voltages (100, 150 and 200 volts) were used. The data were submitted to statistical analysis by SAS statistical software, using a two-way ANOVA, including electroshock voltages (with 3 levels) and time points (with 6 levels) as the main factors. The interaction between the points of analysis and the electroshock intensities was also included in the statistical model.

RESULTS AND DISCUSSION

Behavioral analysis

The use of electroshock was efficient to immobilize fish during desensitization. Some anatomical and physiological characteristics were altered during the desensitization action, such as: bucal musculature propulsion and skin color modification. The cobia has normally black and white stripes next to its lateral line, and the black stripes resulted depigmented during the electricity application.

According to ASPENGREN et al. (2003), a combination of environmental, neural, endocrine and reproductive-related factors influences the mobility of chromosomes in chromatophores and pigment deposition in fish. MOYLE and CECH (1996) emphasized that the color is controlled by the endocrine and nervous systems, so we can infer that the change in the color of cobia skin tissue at the moment of desensitization was due to nervous system's loss of control in relation to the maintenance of pigments state.

Rigor index

This research showed that cobia presented conflicting results comparing to studies done with other species of fish, in which *rigor mortis* was divided in three stages: pre-rigor, full-rigor and post-rigor. In this study the pre-rigor stage was not observed, already in the first evaluation (0 time) the fish being in full rigor, probably as a consequence of the way in which they were transported and stored before the desensitization, or even of the electroshock application, or more probably as a consequence of the synergist action of both. MENDES et al. (2017), using asphyxia and hypothermia after transport, observed that the tambaqui resulted in rigor just after one hour and one hour and fifteen minutes, respectively. In Figure 1, the variations of rigor index in cobia desensitized with different electroshock voltages are shown.

Results of the statistical analysis regarding the rigor index highlighted that there was a significant difference among treatments and that the interaction time×treatment was also significant ($P<0.05$). Among the results related to the treatments it can observed that the 200-volt treatment differed from the others from 24 hours after the desensitization until the end of the period of this parameter evaluation. However, for the unfolding time×treatment, it was observed that there was a significant difference between the point-zero and five hours after the desensitization in all treatments, in relation to the other observation points.

LESSI et al. (1999) in red-tailed brycon (*Brycon cephalus)* (Gunther, 1869) stored on ice, observed that muscle the contraction occurred immediately after desensitization by hypothermia, reaching the total contraction in less than 3 hours after death. These authors cite that the short period of pre-rigor may be characteristic to farmed fish or fish size. ROTH et al. (2012) analyzing the development of rigor mortis in Atlantic salmon subjected to fishing pumping and electrical stunning did not observe significant differences in severity index between fish exposed to 60V, 100Hz AC+DC, while pumping and densification had a significant effect on time during the installation of *rigor mortis*.

The amount of energy consumed by the fish musculature during the desensitization is strictly dependent on the type of current applied, the electric field strength and the current duration (MITTON and MCDONALD, 1994; BARTON and GROSH, 1996). According to ROBB et al. (2003), the electric stunning accelerates the onset of *rigor mortis*, and this can justify what highlighted the fish of the present study.

Rigor mortis is related to the metabolism of muscle glycogen and to the muscle effort and contraction in the moments before the desensitization. When glycogen degradation occurs, the consequent increase of the lactic acid concentration in the muscle is observed, which will lead to a decrease in muscle pH.

pH

According to OCAÑO-HIGUERA et al. (2009) the pH of fish muscle may vary according to species, tissue type, diet, season, activity level or stress during capture. According to POLI et al. (2003) the muscle pH of meagre (*Argyrosomus regius*) shortly after desensitization and slaughter was around 6.97, similar values (6.71) were reported by HERNÁNDEZ et al. (2009). These values are very close to those reported by TEJADA and HUIDOBRO (2002) (between 6.5 and 7.2) in gilthead seabream (*Sparus*

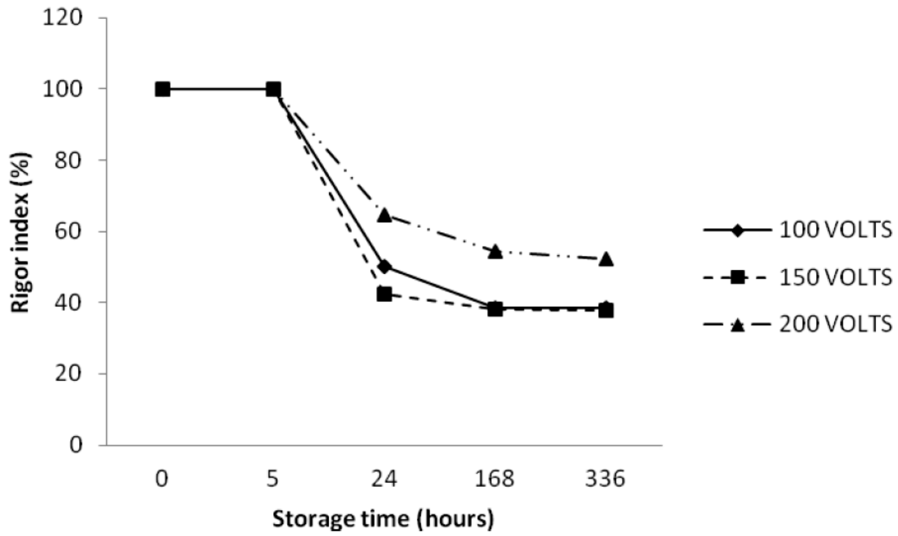

Figure 1 - Variations of the interaction time×treatment for Rigor mortis index in desensitized cobia with different electric shock intensities.

aurata). In the present study, significant differences ($p<0.05$) were found among treatments and among storage times, and the interaction of treatment×time was significant as well, The pH values registered, ranging from 6.02 to 6.66 (Table 1), were well below than the values found in the marine fish of the previous cited papers. In all the treatments investigated, the pH values at 0 time and 5 hours after desensitization and slaughtering were low and this may be related to the pre-slaughter management, diet, or even the applied desensitization method, which accelerates the post mortem biochemistry and the production of lactic acid in the muscle thus decreasing the pH value.

LINES and KESTIN (2005) reported that the greater the intensity of electroshock, the greater the rupture of blood vessels and bloodshed into fish muscle tissue. Fish blood has a lower pH than muscle tissue, and blood shedding may influence muscle pH variation and the microorganism growth. In the present study it was observed that the treatment based on the application of 200 volts for 3 minutes determined the lowest pH value reported a fact that corroborates the statements mentioned by LINES and KESTIN (2005). LAMBOIJ et al. (2006) found pH values close to 7.00 in African catfish using a current intensity of 300-volt, and also observed an immediate fish unconsciousness, without mentioning blood vessel rupture. The pH increase in cobia after 336 and 504 hours of ice storage may be a consequence of the probable bacterial growth, deterioration and formation of nitrogenous substances.

Dielectric properties

According to ÓLAFSDÓTTIR et al. (1997), the freshness changes can be determined by the electric properties of fish muscle. There are limitations of use in terms of fish storage and sample type, and it is not advisable for steaks after a few days in storage. In the present study, there was a marked decrease in the values reported in all treatments (Table 2). There was no significant difference among the treatments ($P>0.05$); however, a significant difference was observed ($P<0.05$) among the storage times. From the results reported it can be inferred that this variable was not influenced by the intensity of the electroshock. TZIKAS et al. (2007) analyzing Mediterranean horse mackerel (*Trachurus mediterraneus*) and blue jack mackerel (*Trachurus picturatus*) specimens found quite high values, respectively, for both the first (85.3-59.0) and the last day (42.4-14.8) of analysis that occurred 12 days after slaughter, when compared to this study, using the same equipment and the same storage form.

Color

The fillets of cobia desensitized with electroshock at different voltages did not present significant difference ($P>0.05$) for color parameters; however, there was a significant difference between the time of evaluation and the time unfolding within treatment ($P<0.05$). The variations of the colors L^* (lightness), a^* (intensity of the red color) and b^* (intensity of the yellow color) are presented in table 3.

In this study, it was observed that with the progression of the storage time, the lightness (L^*) varied in fillets from all the treatments, primarily a slight decrease for treatments 100 and 150 volts, and a sharp decrease for the treatment with 200 volts. After the L^* decrease, there was an increase of this variable, making the fillets more luminous. The intensity of red (a^*) decreased progressively in the treatments with 100 and 150 volts; however, at the end of the storage period, the decrease of

Table 1 - Value variation reported for time×treatment interaction for muscular pH in cobia desensitized with different intensities of electric shock.

	Treatments		
Time (hours)	100V	150 V	200V
0	6.3^{ab}	6.2^{ab}	6.4^{b}
5	6.0^{b}	6.1^{b}	6.3^{ab}
24	6.3^{ab}	6.3^{ab}	6.4^{ab}
168	6.5^{a}	6.3^{ab}	6.3^{ab}
336	6.5^{a}	6.5^{ab}	6.7^{a}
504	6.4^{ab}	6.6^{a}	6.4^{ab}

Lowercase letters indicate differences between times inside the same treatment. (Tukey, 5%).

Table 2 - Dielectric measurements in cobia desensitized with different electric shock intensities.

	Treatments		
Time (hours)	100 volts	150 volts	200 volts
0	13.1^{a}	12.9^{a}	12.9^{a}
5	132^{a}	14.2^{a}	13.5*
24	8.5^{b}	9.4^{b}	8.5^{b}
168	8.7^{b}	9.5^{b}	9.6^{b}
336	7.3^{b}	8.0^{b}	7.9^{b}

Lowercase letters indicate differences between times inside the same treatment. (Tukey, 5%).

the values for all the treatments was observed at the last point of analysis. MORZEL et al. (2002) using electric shock in turbot (*Psetta maxima*) also observed oscillation with a brief decrease followed by an increase both in brightness and intensity of red color, and it is emphasized that the color of the fish fillets was very close using other methods such as percussion. The average values reported in this research are in table 3.

Texture

In the present study, statistical differences were not found for texture characteristics (P>0.05) among samples from different treatments and different storage times. For treatments where the intensities 100 and 150 volts were used, an increase in tissue stiffness is observed, which is not observed in the treatment where 200 volts were used. However, after the 300 hours of storage, flexibilization of the fibers can be observed with the decrease of the force applied to muscular tissue until it ruptures. It is observed here in this study that the texture of the analyzed cobia fillets returned close to the initial value that occurred near 150 hours after desensitization and bloodletting. Texture variations in cobia fillets are shown in figure 2.

Results reported in the present study corroborated the results found by MICHALCZYK and SURÓWKA (2009). According to GODIKSEN et al. (2009) the changes occurred in the fish meat texture are linked to the action of endogenous proteolytic enzymes, and HULTMANN and RUSTAD (2002) reported that the weakening of Z zones in myofibrils is caused by the degradation of connective tissue or even by weakening of actin and myosin bonds.

AZAM et al. (1989) evaluated the texture of rainbow trout (*Salmon gairdneri*) submitted to different desensitization methods (eletronarcosis, carbon dioxide and percussion) and did not find significant differences between samples from the different treatments. OKA et al. (1990) corroborated the results reported by AZAM et al. (1989), in studies carried out in cultured yellowtail killed by different methods.

Sensory evaluation

For the sensory evaluation of cobia kept refrigerated, there was a significant effect of the storage time, whilst the treatments did not differentiate among themselves at each specific storage time (Figure 3). A

Table 3 - Average values for L*, a* e b* colors in cobias desensitized with different electric shock different intensities**.**

---Treatments---									
Time		100V			150V			200V	
(h)		L*			a*			b*	
168	40.2	43.1	42.6	-2.3	-1.5	-2.2	-2.1	-2.6	1.4
336	40.0	40.9	34.1	-2.6	-2.0	-2.1	-4.1	-0.9	-1.0
504	50.2	45.00	48.7	-2.7	-2.6	-2.7	6.9	1.3	2.3
CV(%)		13.11			35.84			48.57	

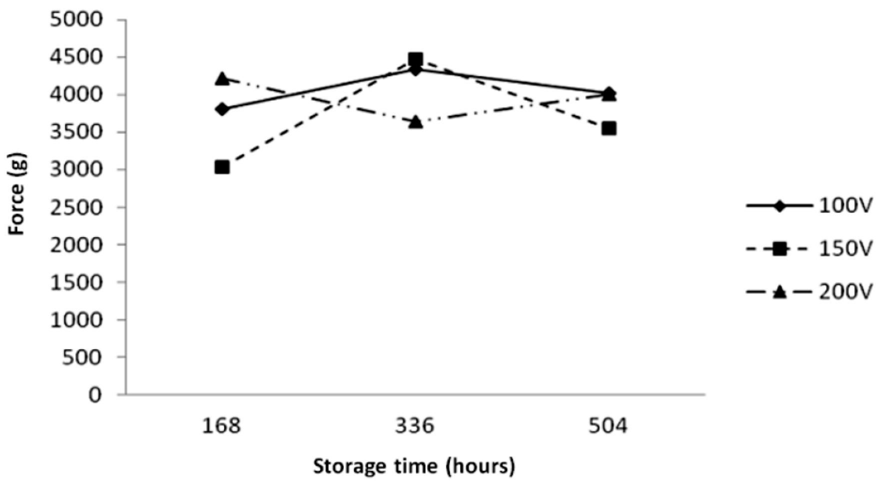

Figure 2 - Variations of the meat texture of cobias desensitized with different electric shock intensities.

marked drop in quality was observed approximately after 350 hours of refrigerated storage, when the samples analyzed reached values close to 1, similar to those reported by PARISI et al. (2002), in researches with sea bass (*D. labrax*). GONÇALVES et al. (2007) found similar variations to those reported in the present study, using hypothermia as a desensitization method.

REGOST et al. (2001) indicated that fish from the marine environment lose their quality from the fourteenth day of storage in ice, which corroborates the results found in this study. According to REGOST et al. (2001), the variation of the values reported for the sensorial evaluation (Figure 3) also depends on the fat content of the fish, which influences the storage time.

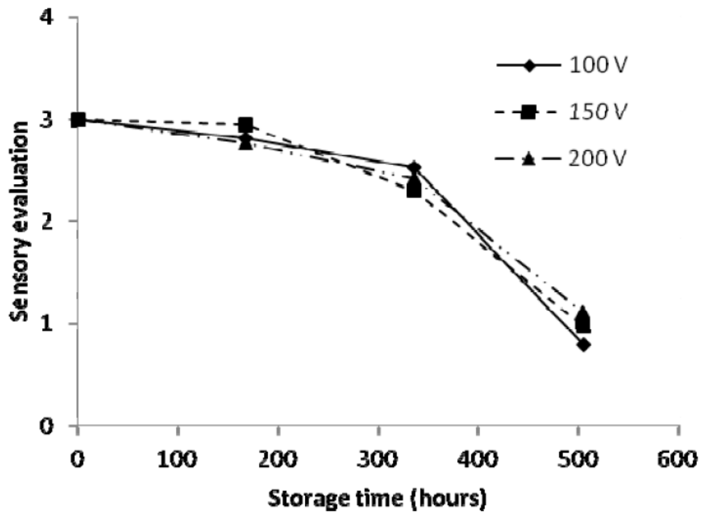

Figure 3 - Variations of the observed values of the interaction time×treatment for sensory evaluation of cobias desensitized with different electric shock intensities.

ATP and its catabolites

The ATP (adenosine triphosphate) degradation is caused by energy consumption and bacteria activity. After the desensitization process, *rigor mortis*, a phenomenon derived from muscle contraction after death, is observed. During muscle contraction there is energy consumption in the form of ATP, which must be degraded in the chain producing various catabolites from ATP that help for the characterization of fish quality.

Differences were observed for storage times in all studied variables; however, differences between the treatments were observed for IMP (inosine monophosphate), ATP (adenosine triphosphate), ADP (adenosine diphosphate), AMP (adenosine monophosphate) and Hx (hypoxanthine) (Figure 4). There were proportional decreases between the ATP and its metabolites, a fact that can be observed inversely for the pH and *rigor mortis* variables. For the ATP and IMP variables, MORKORE et al. (2010) observed values higher than results reported in this study (0.5 and 9.3μmol g^{-1}, respectively), evaluating Atlantic salmon in the first 24 hours after desensitization. According to MORKORE et al. (2010) the variations of IMP may be due to the action of autolytic enzymes, and their variations in seasonal enzymatic concentrations. According to HOWGATE (2006) the mean values of IMP reported in rainbow trout surveys are close to 6.8μmol g^{-1}.

The ATP values decreased according to the increase in the storage period, until this variation becomes almost constant; however, the opposite is observed for the AMP nucleotide, precisely at the end of *rigor mortis*. The variations reported for ATP, ADP, AMP and IMP are in figure 4.

Concentrations of inosine and hypoxanthine reported in cobia do not show equivalent proportions, with hypoxanthine being found in higher concentrations. Among the variations found it can be observed that after 168 hours of storage the contents of Hx and HxR (inosine) are quite different, and the intensity at 200 volts showed the lowest values.

Maximum values of hypoxanthine did not exceed 1.06μmol g^{-1} in the 336th hour of storage, for the intensity in 150 volts. From the obtained results, it can be inferred that the hypoxanthine concentrations increased according to the storage time for all the treatments, being the treatments different ($p<0.05$). Observing results reported for the value K (Figure 5), it can be inferred that the treatments with intensities

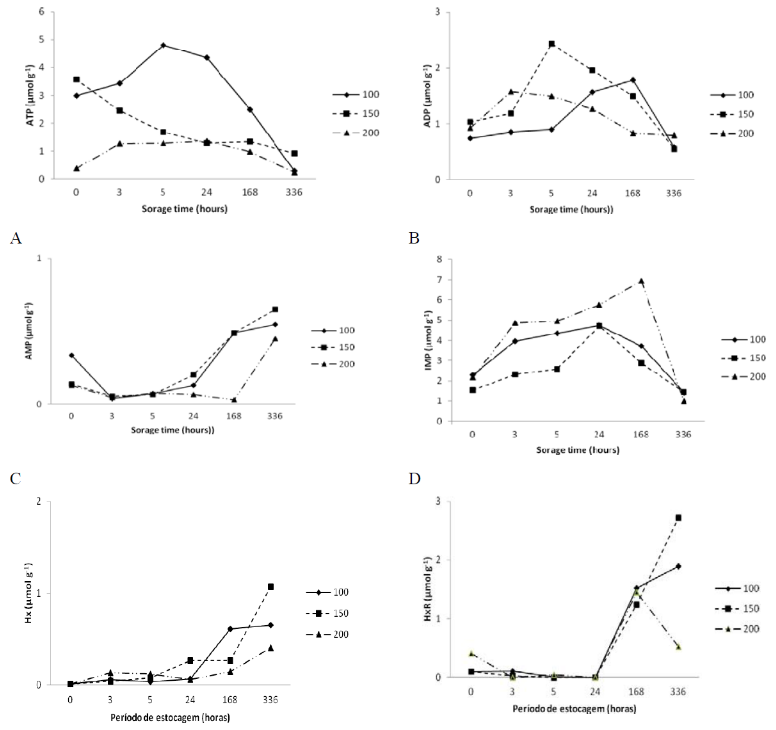

Figure 4 - Changes in concentration of ATP (A), ADP (B), AMP (C), IMP (D), Hx (E), HxR (F) in cobia desensitized with different electroshock intensities.

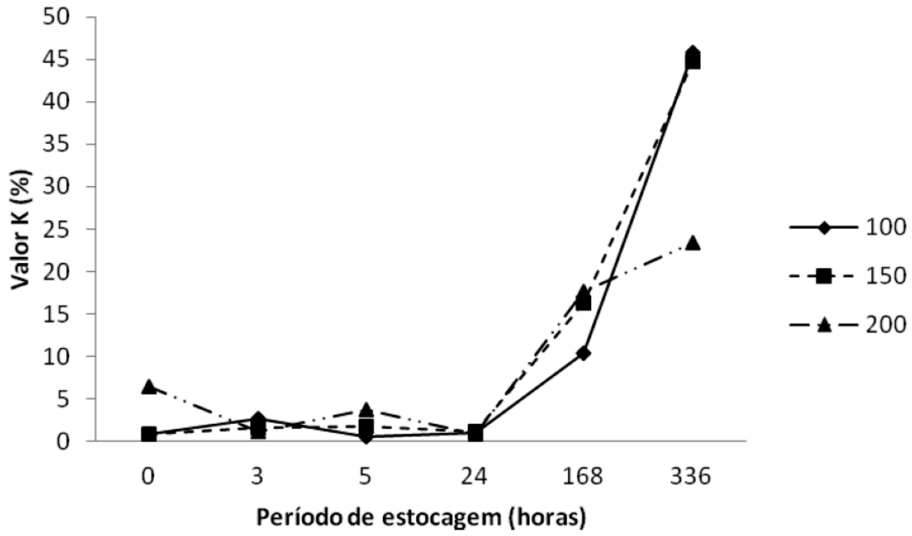

Figura 5 - K value variations in cobias desensitized with different electric shock intensities.

in 100 and 150 volts showed a marked increase from the 168th hour of storage, surpassing 40%, which allows concluding that the fish were not suitable for consumption (DELBARRET-LADRAT et al., 2006).

CONCLUSION

The use of electroshock was efficient with regard to immobilization of fish during desensitization.

The electrical current of 150 volts applied for the desensitization of cobia provided better quality results over meat storage time. Through the observed results, the 200-volt intensity is not recommended to numb the cobia; because, numerous ruptures of veins and arteries occur, damaging the quality of the meat during the storage.

Finally, by the results obtained it can be affirmed that this species can be stored under refrigeration for a 7-day period after slaughter, maintaining a high quality condition when desensitized by the methods investigated.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Ruling of the Ethics Committee of USP Nº 2012.1.337.742.2, APPROVED on 05/16/2012.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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