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7 Biogenic amines assessment during different stages of the
8 canning process of skipjack tuna (*Katsuwonus pelamis*)
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ABSTRACT

The present research focused on the biogenic amines (BAs) formation in skipjack tuna (*Katsuwonus pelamis*) throughout the whole canning process. In agreement with its wide employment on this species, on-board brine-immersion freezing (BIF) was tested as post-mortem processing. The study included fish samples corresponding to different stages of the canning process such as frozen, thawed, cooked and canned; after cooking, two kinds of tuna muscles were considered, i.e., whole fillets (main product) and grated muscle (off-product arising from small pieces). For the BAs (tryptamine, putrescine, cadaverine, histamine, spermidine and spermine) assessment, a HPLC-DAD method was developed and validated in skipjack tuna samples, in agreement with different parameters such as suitability, linearity, limits of detection and quantification, precision, accuracy and robustness. Tuna submitted on-board to BIF procedure provided low levels of spermine and spermidine (up to 27.6 mg kg⁻¹), while contents on the remaining BAs maintained below the limit of detection. Throughout the different stages of the canning process, skipjack tuna showed a low formation of most BAs; interestingly, histamine content was found below 10.6 mg kg⁻¹ level. The highest values were obtained for spermidine, these related to cooked grated tuna (from 22.6 to 66.7 mg kg⁻¹) and canned grated tuna (from 70.6 to 104.4 mg kg⁻¹). Values for pH assessment in all kinds of tuna samples corroborated the results obtained for BAs determination. BIF procedure proved to be an amenable post-mortem processing to guarantee the quality of canned skipjack tuna.

Key words: HPLC, canning, food safety, chilling, fish, fish toxins, physical preservation methods, food processed

Running title: Biogenic amines in canned skipjack tuna

INTRODUCTION

Marine species give rise to excellent canned products and support a great market demand. From the moment they are caught till they are ready to be canned, marine species are submitted to varied handling and technological stages (Horner, 1997; Aubourg, 2001). Consequently, detrimental changes have been reported during such stages, so that nutritional, sensory and safety values of the final canned product can be negatively modified (Izquierdo *et al.*, 2007; Kim *et al.*, 2009; Prester, 2011; Lucci *et al.*, 2016). To preserve the quality of marine species to be further employed for canning, different strategies are employed. Among them, brine-immersion freezing (BIF) has widely been employed on-board after capture by large fishing vessels and by tuna canneries; in it, immersion in an aqueous solution with a low freezing point would lead to a fast freezing of fish pieces, this allowing the storage of huge quantities of raw material (Aubourg & Gallardo, 2005; Bodin *et al.*, 2014). The preservative effect of salt has been attributed to less availability to microbial attack, enhancement of functional properties and decrease of water activity (Chiralt *et al.*, 2001). In spite of these advantages, the application of the method may undergo temperature variations and negative effects during handling; as a result, physical, chemical and microbiological changes may occur, these leading to microbial activity and lipid oxidation development (Aubourg & Ugliano, 2002; Bodin *et al.*, 2014; Restuccia *et al.*, 2015). Aquí creo q está mal citado

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Biogenic amines (BAs) are compounds formed in food by decarboxylation of free amino acids as a result of amino acid decarboxylase enzyme activity and are indicators of microbial degradation. The most common monoamines in fish are histamine (HIS) and tryptamine (TRY), which are produced from histidine and tryptophan, respectively. Additionally, polyamines such as putrescine (PUT) and cadaverine (CAD), arising from ornithine and lysine, respectively, as well as spermine (SPM) and spermidine (SPD), both arising from putrescine, have also attracted a great attention (Önal, 2007; Koral *et al.*, 2013;

Alvarez & Moreno-Arribas, 2014; Tofalo *et al.*, 2016). BAs formation has been reported to depend on factors such as the free amino acid content, microbial activity development, and accurately of processing and preservation conditions during the post-mortem period (Elsanhoty & Ramadan, 2016; Mohammed *et al.*, 2016; Restuccia *et al.*, 2015 aquí mejor).

The levels of BAs can also increase in processed such as salting, ripening, fermentation or marination (Visciano *et al.*, 2012). Among BAs, histamine presence is known to be a major concern in seafood safety (Chen *et al.*, 2010; Prester, 2011). Its formation occurs especially in the *Scombridae* fish family, and may lead to the so-called “*Scombridae poisoning*” (Mohan *et al.*, 2015).

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Other biogenic amines, although not implying a direct risk, can also have negative effects on health. This is the case of putrescine and cadaverine that may favor the histamine toxicity, while putrescine, cadaverine, spermidine and spermine have been reported to be potentially carcinogenic (Kim *et al.*, 2009; Park *et al.*, 2010; Alvarez & Moreno-Arribas, 2014; Tofalo *et al.*, 2016).

Related to BAs assessment, most efforts have been directed to histamine detection (López-Sabater *et al.*, 1994; Gallardo *et al.*, 1997). However, and in agreement with the great incidence of other BAs on safety, several analytical methods have been proposed for the simultaneous determination of most BAs in seafood (Park *et al.*, 2010; Köse *et al.*, 2012; Sánchez & Ruiz-Capillas, 2012). Among them, the HPLC separation by employing a reverse-phase column, followed by quantification with a photodiode array detector (DAD) has widely been used, as providing high precision and resolution (Park *et al.*, 2010). Such procedure would avoid the employment of detectors with higher sensitivity, but more expensive such as MS or MS/MS, still applied with long separation time and for specific food matrices (Erim, 2013).

Skipjack tuna (*Katsuwonus pelamis*) is among the most important fish species for the Global market. More than 3 million tons are caught each year mainly intended for the

canning industry of this *Scombridae* species (FAO, 2016). Previous research accounts for the BAs formation in skipjack tuna during post-mortem storage (Rossi *et al.*, 2002; Staruszkiewicz *et al.*, 2004) as well as in the commercial canned product (Sims *et al.*, 1992; Veciana-Nogués *et al.*, 1997). However, research concerning the effect of preliminary processing on canned product quality can be considered scarce (Jeya Shakila *et al.*, 2005). The present research focused on the BAs formation in skipjack tuna throughout the whole canning process. In it, on-board BIF procedure was applied and a HPLC-DAD analytical method was developed and validated for tuna samples.

MATERIALS AND METHODS

Standards and reagents

Stock solutions of the various biogenic amines (tryptamine hydrochloride, histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride) (Sigma-Aldrich Co, Saint Louis, MO, USA) were prepared separately in the concentration of 3.0 g L⁻¹ in 0.1 M HCl. Working solutions (0.3 g L⁻¹) were prepared by diluting the stock solution with 0.1 M HCl and used to obtain the calibration curves. Both the calibration and matrix curves were prepared with the following concentrations: 3, 6, 9, 12, 18, 24 and 30 mg L⁻¹.

1,7-diaminoheptane (Sigma-Aldrich Co) was employed as quantitative internal standard (IS). Its stock solution was prepared by dissolving 20 mg in 100 mL of 0.1 M HCl. The working solution was prepared by diluting the stock solution to a 4.0 mg L⁻¹ concentration with 0.1 M HCl.

Dansyl chloride was employed for BAs derivatization. For it, a 10 mg mL⁻¹ solution was prepared by dissolving 100 mg in 10 mL of acetone.

All solutions were stored in the dark at 4 ± 1 °C prior to use.

Raw fish, sampling and canning process

The present study was carried out in three complementary parts. In the first one, the validation method for BAs assessment was developed. In the second part, such method was applied to skipjack tuna samples being removed from on-board, and in the third step the method was applied in different stages of the canning process. At all parts, skipjack samples (average weight of 7.4 kg, included in the 3.1-11.3 kg range) were caught by rod and live bait on the coast of Santa Catarina (latitude 19-34 °S, longitude 40-50 °W) in the West Tropical Atlantic Ocean and on-board freeze by brine immersion (-8.2 ± 3.8 °C). Brine-freeze samples were obtained at the Itajaí port (SC, Brazil) and transported to industry where canning was carried out.

For the first and second part of the study, 24 frozen individuals were employed. Among them, 22 had been freeze on-board by brine immersion (on-board brined tuna, OBT condition), while 2 individuals had been frozen under traditional condition (stored in a common freezer at -12 °C; control frozen tuna, CFT condition).

For the first and third part of the study 54 fish samples were taken into account and distributed into six different stages of the skipjack tuna canning process. Such stages were the following: frozen brined tuna (FBT condition; fish arrived at the industry kept frozen for 16 days at -20 ± 0.5 °C), thawed tuna (TT condition; fish thawed at 15-21 °C), cooked solid tuna (CST condition; cooked whole muscle tuna), cooked grated tuna (CGT condition; cooked grated muscle tuna), canned solid tuna (CANST condition) and canned grated tuna (CANGT condition). After thawing the fish in tanks for 4 hours, cooking was carried out at 90 °C for 3 hours. After cooking, two kinds of cooked tuna were considered, i.e., whole fillets (main product) and grated muscle (off-product). Whole fillets are obtained from the whole loin of tuna, while the grated sample comes from the pieces of muscles that are more adhered to the backbone and have to be separated mechanically in small pieces. Both kinds of cooked muscle were considered separately and placed in cans with a total weight of 170

g, this also including water, salt, and vegetables (soy, carrot, and celery). Cans were closed and sterilization was carried out at 118 °C for 42 min.

Each stage was evaluated in three independent lots (n=3), each lot including 3 fish individuals that were pooled together for analysis.

All samples were storage in bags and transported in ice to the laboratory within 3 h. Upon arrival, samples were immediately subjected to BAs analyses or frozen at -18 ± 2 °C and defrosted at the refrigerated temperatures prior to analysis

Biogenic amines extraction and derivatization

BAs were extracted as described by Park *et al.* (2010) with some modifications. Thus, 10 g of minced muscle of each sample were transferred into a centrifuge tube containing 13.8 mL of 6% (w/v) trichloroacetic acid (TCA) and 2 mL of 0.1 M HCl, vortexed for 30 sec, and placed in a Unique 1400A ultrasonic bath (Unique, São Paulo, Brazil) at 25 °C for 16 min. The mixture was then centrifuged at 3250xg for 15 min and the supernatant filtered through a Whatman (N° 1) paper. Then, 1 mL of each extracted sample or 1 mL of the standard solution of any biogenic amine was mixed with 0.1 mL of the IS, 200 µL of sodium hydroxide (2.0 M) and 300 µL of saturated sodium bicarbonate (780 mg mL⁻¹) and vortexed for 20 s. Under this alkaline condition, the mixture was derivatized with 2 mL dansyl chloride by incubation for 45 min at 40 °C. After that time, 100 µL of L-proline (100 mg mL⁻¹) were added to remove any residual dansyl chloride. The mixture was then incubated for 15 min at room temperature in the dark. Finally, 1.4 mL of acetonitrile was added to the solution, centrifuged at 3000xg for 15 min, and the supernatant was injected into chromatographic system.

Determination of biogenic amines

The BAs analysis was carried out by the HPLC (1260 Infinity Quaternary LC, Agilent Technologies, Palo Alto, CA, USA), with a DAD detector set at 254 nm and equipped with an auto sampler with an injection volume of 2 μ L, as described by Park *et al.* (2010) with modifications. Separation was performed on a Zorbax Eclipse plus C₁₈ column (100 mm length, 3 mm internal diameter, and 3.5 μ m particle size; Agilent Technologies) with 50 x 3 mm security-guard column, temperature control set at 40 °C and data treatment software (HP ChemStation, Palo Alto, CA, USA). Mobile phases, which were vacuum filtered and degassed prior to use, were composed by deionized water and acetonitrile gradient elution as detailed in Table 1. Amines identification was based on the comparison of the retention times of standard solutions and spectral characteristics. The levels of amines in the samples were determined by interpolation from external calibration curves constructed with standard solutions in a brined matrix, i.e., by representing the peak area ratios (analyte/IS) versus analyte concentration for the six different BAs. Analyses were carried out in triplicate.

Method validation

In order to verify the method performance, system parameters such as suitability, linearity, matrix effects, limit of detection (LOD) and quantification (LOQ), precision (intra-day and inter-day), accuracy and robustness were evaluated (Eurachem Working Group Eurachem, 1998).

Assessment of the pH value

The pH measurements were done with a portable pH-meter (HI 99163N, Hanna, Brazil) according to the AOAC 981.12 procedure (1990). For it, the electrode was inserted 5 cm at 20 °C on the fish muscle corresponding to the different stages of the canning process taken into account. Analyses were carried out in triplicate.

Statistical Analysis

The experiments were carried out in triplicate ($n = 3$). Thus, data were expressed as the mean of three independent determinations. Data were subjected to one-way analysis of variance (ANOVA) and the means were compared by the Tukey test and F -test at 5% probability using the software Statistica 7.0 for Windows (Stat Soft Inc., USA).

RESULTS AND DISCUSSION

Development and validation of the method

As a first task, the behavior of the various biogenic amines was analyzed by HPLC by employing their corresponding commercial standards. Once separated and identified (Fig. 1, panel a), tuna samples corresponding to the second part of the study were injected, separated and identified. As a result, tuna samples corresponding to control frozen tuna (CFT; panel b) provided the same profile as frozen brined tuna (FBT; panel c). Unknown peaks were evident in both kinds of samples and were marked with an asterisk (*). In agreement with Fig. 1, BAs were separated with a total run time of 12 min with good peak resolution, sharpness, and symmetry.

As a following task, the validation of the method was undertaken. The system suitability was checked from 10 consecutive injections of the standard solution. Thus, the corrected peak area (A') (analyte area/IS area) and the corrected migration time (t_R') (analyte time/IS time) showed relative standard deviations (RSD) from 0.11% to 0.83%, so that the instrumental system was found suitable to be used in the validation process (data not shown).

Linearity was evaluated by using seven equally-spaced concentration levels of standard solutions (concentrations of 3 - 30 mg L⁻¹) for each amine; this analysis was carried out in triplicate. Linear calibration curves were constructed by representing the peak area ratio (analyte/IS) versus the analyte concentration for each amine and rated as the correlation coefficient (R²). Results showed good linearity, with R² being higher or equal than 0.9999 in all BAs (Table 2).

Linearity was confirmed by the ordinary least-square method (OLSM) that analyzes the plots and residual calculations (Souza & Junqueira, 2005). In this method, the presence of outliers was evaluated by the Grubbs test, and the residuals were found to be normally distributed (considering $p < 0.05$) in agreement with the Shapiro-Wilk test. Residuals were homoscedastic, according to the Cochran test, and demonstrated a remarkable adjustment to the model (F -test at 5%) in agreement with the Durbin-Watson test.

Matrix effects were evaluated through the F -test and t -test in tuna samples (Table 2). For it, comparison of the slopes of both standard solutions and matrix calibration curves, which also quantified the R² and OLSM, was carried out (Thompson *et al.*, 2002). Thus, a matrix effect was observed for tryptamine and histamine because the responses of the curved slopes were significantly different at a 95% confidence level ($p < 0.05$). Accordingly, matrix calibration curves must be used for the quantification samples in such cases. The curve indicated linearity ($R^2 \geq 0.99$), which was confirmed by the OLSM that displayed no outliers, residuals were normally distributed, homoscedastic, independent and adjusted to the model (F -test at 5%).

The LOD and LOQ were determined for the tuna samples, calculated based on signal/noise ratios of 3:1 and 10:1, respectively. These limits were established based on the mean obtained from 10 independent replicates. Values obtained were found similar to those of the previous reported literature (Dadáková *et al.*, 2009; Saaid *et al.*, 2009) and demonstrated a high detectability of the method. LOD values for the six BAs studied were

between 0.05 ± 0.01 and 0.24 ± 0.01 mg kg⁻¹, while the LOQ values were from 0.18 ± 0.01 to 1.23 ± 0.01 mg kg⁻¹ (Table 2). These results indicate that the method is suitable for application to canned samples.

The precision was determined by three injections of three concentration levels in three independent replicates by evaluating inter-day (3 days) and intra-day (n=3) variations. For it, the peak area ratio (analyte area/IS area) was taken into account and the migration time was corrected (time analyte/time IS). As a result, the inter-day average precision for BAs was 2.31 % RSD and the intra-day precision was 0.87 % RSD.

Accuracy of the analytical method was determined by apparent recovery obtained from the mean for the three independent replicates of spiked on-board brine tuna (OBT), canned solid tuna (CANST) and canned grated tuna (CANGT) samples at three concentration levels (low, medium and high levels; concentrations of 6, 12 and 24 mg L⁻¹, respectively) and expressed as % recovery.

Results showed to be ranged between 70.7% (histamine) and 119.8% (cadaverine) (Table 3). Amine values were found similar to the ones reported in fish products by Chen *et al.* (2010) (from 55.6 to 109.7%) and by Park *et al.* (2010) (from 84 to 95%). Consequently, accuracy of the current analytical method was proved.

The robustness was evaluated through the Youden test, by evaluating the performance parameters A', t_R', concentration, symmetry and resolution, when small changes were applied in different kinds of parameters included in the analytical procedure such as vortex extraction time, derivatization temperature, derivatization time, time in the dark, vortex derivatization time, centrifugation time and stability time. The method was found to be robust because the performance of parameters showed values lower than 1.3% (data not shown).

All these data showed that the sample preparation and quantification procedures were both reproducible and reliable. Therefore, the HPLC method proposed in the current

study could be considered as a useful tool for the quantification of biogenic tuna samples corresponding to different stages of the canning process.

Evaluation of frozen tuna samples corresponding to the on-board immersion condition

Tuna samples remained immersed on-board in seven brine tanks between 10 and 26 days with tank temperatures showing great temperature variability, thus ranging from -18.1°C to 22.6°C (average value of $-8.2 \pm 3.8^{\circ}\text{C}$). In this regard, a lower temperature than -9°C has been recommended by the European Commission (EEC, 2003) for brined fish.

Despite the different immersion times and temperatures applied for the freeze storage, the levels of all BAs (Table 4) in the on-board brined tuna (OBT) samples were low (i.e., between $<\text{LOD}$ and 27.6 mg kg^{-1}). Concerning histamine, low values were detected in all cases ($<\text{LOD}$), which showed not to be produced as a result of the brining process and storage under such condition; histamine content was found lower than legal limits (50 mg kg^{-1}) established for fish products by the US Food and Drug Administration (FDA, 2011), and also lower than 100 mg kg^{-1} established by the European Commission (EEC, 2003) and by the Brazilian Ministry of Agriculture and Livestock (Ministério da Agricultura, Pecuária e Abastecimento, Portuguese acronym MAPA) (Brasil, 1997). Additionally, on-board brined tuna (OBT) revealed no significant differences ($p>0.05$) in levels of biogenic amines as compared to the control frozen tuna (CFT), thus demonstrating that the brine-immersion freezing represents an efficient method for maintaining the on-board skipjack tuna quality in relation to the BAs formation (Koral & Köse, 2012).

Application of the method to tuna samples corresponding to different stages of canning

The content of BAs evaluated during different stages of the tuna canning process is presented in Table 54. Results corresponding to each lot are expressed separately, so that

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comparison among lots and changes of each lot throughout different canning stages can be pointed out.

The tryptamine content was not found to be influenced by the canning process. Thus, its content was found in all cases below the LOD. Such content agrees with the one obtained for skipjack in nature (Kim *et al.*, 2009), and even with values found by Park *et al.* (2010) in canned tuna (from <LOD to 10.1 mg kg⁻¹).

In spite of revealing a low level, some formation was found in lot 1 for putrescine in cooked grated tuna (1.6 mg kg⁻¹). After the cooking process, handling and the mechanical separation of thorns take place that may facilitate the formation of some BAs (Koral & Köse, 2012); consequently, this may be the explanation for the formation, in this case, of putrescine. After canning (CANST and CANGT stages), putrescine levels decreased because this biogenic amine is an intermediate in the synthesis of spermidine and spermine (Glória, 2005). Lower levels of putrescine than 0.9 mg kg⁻¹ have been reported for fresh tuna (Koral & Köse, 2012).

Formation of cadaverine was obtained, especially after the cooking process. Values, however, can be considered as low, since the highest value (i.e., < 9.6 mg kg⁻¹) was obtained for CGT samples in lot 1. As for putrescine, the increase of cadaverine content after filleting can be explained on the basis of contamination during the handling and the mechanical separation of thorns; this effect was found higher in grated samples than in solid ones. A marked decrease of the cadaverine content was observed at the canned stages. Thus, both for solid and grated canned samples, a marked cadaverine content decrease were implied in all lots under study. Biogenic amine compounds are known to be heat-resistant; consequently, freezing, cooking and sterilization would not affect their content. Contrary, their content decrease can be related to being extracted by the coating medium in the can, and thus be eliminated prior to analysis of the canned muscle (Prester, 2011). Concerning acceptance of canned fish, previous research proved a direct relationship between the

putrescine and cadaverine contents and the sensory values attributed to commercially canned skipjack tuna (Sims *et al.*, 1992); both BAs were found valuable to settle a *cut-off* point before rejection of the product. Additionally, cadaverine showed to be formed prior to and/or accumulated at a faster rate than histamine during refrigerated storage of skipjack tuna (Rossi *et al.*, 2002); it was concluded that cadaverine, either alone or with histamine, could be used as an index of decomposition for skipjack tuna.

Formation of histamine was depicted in lots corresponding to both kinds of cooked samples (CST and CGT); however, values were in all cases low (between $< \text{LOD}$ and 10.6 mg kg^{-1}). Such levels can be considered in agreement with Mohan *et al.* (2015) who found an increase of 8.2 to 12.7 mg kg^{-1} during cooking (120°C) of canned tuna. This increase was explained on the basis of an increased activity of bacterial enzymes attributed to the elapsed time before the cooking process takes place; such delay would facilitate the degradation of histidine into histamine by microbial activity development (Kung *et al.*, 2009; Silva *et al.*, 2011). Contamination of tuna fish (*Thunnus thynnus*) was also found during capture and subsequent unhygienic handling in the canning plant (López-Sabater *et al.*, 1994); thus, histamine-former bacteria were identified in tuna muscle, although histamine content remained in all cases as acceptable. Delayed processing during skipjack canning also reflected marked histamine content increase (Jeya Shakila *et al.*, 2005); however, values did not overpass the acceptable level. The effects of on-board and dockside handling on the formation of histamine in skipjack tuna were studied by Staruszkiewicz *et al.* (2004). It was observed that at 26°C , more than 12 hours of fish incubation were necessary before histamine concentration of 50 ppm was reached, while at 35°C , 50 ppm histamine formed within 9 hours. Besides, all samples demonstrated limits lower than the legal for histamine. The content in canned tuna ($< \text{LOD}$) was lower than 12.7 mg kg^{-1} as discovered by Mohan *et al.* (2015), to 42.9 mg kg^{-1} by Izquierdo *et al.* (2007), and 9 mg kg^{-1} by Jeya Shakila *et al.* (2005).

Spermidine showed the greatest contents of all biogenic amines throughout the whole canning process. Its formation was already observed in two lots of frozen brined tuna, increased in most thawed samples, and provided great variable contents in cooked and canned samples. The most important trend to be concluded is the fact that grated samples provided higher values ($p < 0.05$) than their corresponding solid samples in nearly all cases. As for other biogenic amines, this difference can be originated after the filleting process, which involves handling and mechanical separation of thorns. This presence of spermidine in canned samples can be considered as a concern, since this biogenic amine when combined with a nitrite, a substance present in plant extracts, can lead to the formation of nitrosamines, known to be carcinogenic (Kim *et al.*, 2009; Moret *et al.*, 2005). According to the results obtained, this problem would be markedly more important in the case of grated canned samples than in their corresponding solid ones. In this regard, current legislation ought to be developed for other biogenic amines than in the case of histamine (Sánchez & Ruiz-Capillas, 2012).

Finally, polyamine spermine has shown low levels in all stages, being values obtained for all kinds of canned samples under the limit of detection. As previously explained, its content ought to decrease as a result of being extracted by the dipping medium of the can. Both spermine and spermidine are produced by agmatine breaking; interestingly, its formation would not decrease by inactivating the corresponding decarboxylase enzymes (Köse *et al.*, 2012). Interestingly, previous research showed that the content of both spermidine and spermine would be high in fresh tuna and in canned tuna (Veciana-Nogués *et al.*, 1997); contrary, no effect on other BAs was found between the fresh and canned stages.

Because histamine is not solely responsible for scombroid poisoning, but act synergistically with other amines, Mietz and Karmas (1977) proposed the quality index (QI) to evaluate the decomposition of tuna fish. Furthermore, this index showed to be adequate

for the evaluation of the quality of fish and seafood in general (Bakar *et al.*, 2010; Zare *et al.*, 2015). As proposed, the QI can be defined, or calculated according to the following equation:

$$QI = (PUT + CAD + HIS) / (1 + SPD + SPM)$$

In such equation, PUT, CAD, HIS, SPD and SPM denote the contents (mg kg⁻¹) on putrescine, cadaverine, histamine, spermidine and spermine, respectively. By applying this equation, a QI included in the 0-1 range would indicate good quality, while a score higher than 10 would correspond to rejectable quality (Oliveira *et al.*, 2012). In the current study, despite some BAs formation in the different canning stages, all QI values were included in the 0.0-2.2 range, so that good quality can be concluded in all cases.

Assessment of the pH value of tuna samples corresponding to different stages of canning

Changes in the pH value of skipjack tuna muscle throughout the canning process are shown in Table 65. Skipjack individuals were frozen immediately after being captured, previously to the *rigor mortis* period setting. After thawing, a general decrease of average pH values was observed, this decrease being significant (p<0.05) in the case of lot 2. Such a decrease can be explained on the basis that glycogen breakdown into lactic acid occurred during this period (Bahmani *et al.*, 2011).

The pH achieved was lower than those found in *Thunnus thynnus* tuna (Selmi & Sadok, 2008) (6.3 value), and higher than in *Thunnus obesus* tuna (5.4 value; Ruiz-Capillas & Moral, 2005). Differences can be attributed to several factors such as fish species, microbial type and load, handling and storage conditions and slaughtering stress (Aursand *et al.*, 2010; Chaijan, 2011). An additional factor to take into account would be the preservation method applied in the present case for the freezing process. Thus, brine

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freezing would facilitate the salt diffusion into the tuna muscle, this leading to lower pH values causing the salt to bind to the protein (Larsen *et al.*, 2008; Chaijan, 2011).

No relevant changes could be observed in all lots as a result of cooking and sterilization, both in solid as in grated samples. Values in all cases can be considered as low, being kept below a 6.1 score. Values were in agreement with the maximum level (pH=7.0) requirements of the Regulation of Industrial and Sanitary Inspection over Products of Animal Origin - RIISPOA accepted in Brazil (Huss, 1995; Brasil, 2017).

CONCLUSIONS

An HPLC-DAD method was developed and validated for the BAs assessment throughout different stages of the canning process of a greatly important tuna species. Despite the time and nonconforming temperatures commonly developed during on-board storage after BIF, BAs levels remained at low concentrations without significant differences when compared with the control frozen tuna samples. Thus, tuna submitted on-board to BIF procedure provided low levels of spermine and spermidine (up to 27.6 mg kg⁻¹), while contents on the remaining BAs maintained below the limit of detection. Throughout the different stages of the canning process, skipjack tuna showed a low formation of most BAs; interestingly, histamine content was found below 10.6 mg kg⁻¹. Interestingly, the highest values were obtained for spermidine, these related to cooked and canned samples corresponding to grated tuna, an off-product resulting from the canning process; consequently, a special attention ought to be accorded to the formation of this amine. However, in all kinds of tuna samples, values for QI and pH corroborated the results obtained for individual BAs determination. As a result, BIF procedure proved to be an amenable post-mortem processing to guarantee the quality of canned skipjack tuna.

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TABLE 1

HPLC gradient condition for the quantification of dansylated biogenic amines

Time (min)	Deionized water (%)	Acetonitrile (%)	Flow (mL min ⁻¹)
0.0	40	60	0.4
1.0	40	60	0.4
2.0	40	60	0.5
3.0	25	75	0.5
4.0	25	75	0.5
6.0	5	95	0.5
8.0	5	95	0.5
10.3	40	60	0.5
12.0	40	60	0.4

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TABLE 2

Validation parameters of the HPLC method*

BAs	Linearity				Precision intra-day (% RSD)		Precision inter-day (% RSD)	
	Linearity	Matrix effect	LOD**	LOQ**	A'	t _R '	A'	T _R '
Tryptamine	1	0.9999	0.18 ± 0.04	0.36 ± 0.10	0.87	0.32	5.87	0.20
Putrescine	1	0.9994	0.05 ± 0.01	0.21 ± 0.02	1.50	0.11	3.03	0.05
Cadaverine	1	0.9997	0.06 ± 0.01	0.18 ± 0.01	1.23	0.12	1.16	0.07
Histamine	0.9999	0.9931	0.20 ± 0.03	0.38 ± 0.04	1.98	0.06	6.15	0.03
Spermidine	1	0.9982	0.23 ± 0.01	1.23 ± 0.01	1.94	0.11	4.54	0.09
Spermine	1	0.9988	0.24 ± 0.01	0.63 ± 0.02	2.08	0.08	6.49	0.07

630 * Abbreviations employed: LOD (limit of detection; n=10), LOQ (limit of quantification; n=10), RSD (relative standard deviation; n=9), A' (corrected
631 peak area; n=9) and t_R' (corrected migration time; n=9).

632 ** Values expressed as average ± standard deviation (mg kg⁻¹ muscle).

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TABLE 3

Accuracy (%) of biogenic amines assessment in various spiked skipjack tuna samples*

Biogenic amine	Spiked concentration								
	6 mg kg ⁻¹ muscle			12 mg kg ⁻¹ muscle			24 mg kg ⁻¹ muscle		
	OBT	CANST	CANGT	OBT	CANST	CANGT	OBT	CANST	CANGT
Tryptamine	80.5	83.5	74.6	79.8	72.3	73.5	77.9	78.7	73.6
Putrescine	108.2	110.4	96.4	103.8	98.6	95.8	102.0	102.3	96.6
Cadaverine	113.3	119.8	111.1	110.9	111.1	111.3	109.8	116.8	110.7
Histamine	70.7	104.5	100.9	72.2	88.8	98.9	73.4	97.4	99.1
Spermidine	82.3	112.6	92.0	85.4	72.9	90.3	85.8	88.8	93.1
Spermine	83.9	94.1	88.1	75.6	88.0	90.0	71.4	94.1	88.1

* Tuna samples abbreviations: OBT (on-board brined tuna), CANST (canned solid tuna) and CANGT (canned grated tuna).

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TABLE 4

Con formato: Resaltar

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Biogenic amines (mg kg⁻¹ muscle) and quality index* in on-board brined tuna and control frozen tuna samples** conserved on-board

Sample	Lot	Biogenic amines						Quality Index
		Tryptamine	Putrescine	Cadaverine	Histamine	Spermidine	Spermine	
OBT 1	1 (n=7)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 2		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 3		<LOD	<LOD	<LOD	<LOD	0.3	<LOD	0.0
OBT 4		<LOD	<LOD	<LOD	<LOD	7.6	<LOD	0.0
OBT 5		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 6		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 7		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 8	2 (n=5)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 9		<LOD	<LOD	<LOD	<LOD	<LOD	17.5	0.0
OBT 10		<LOD	<LOD	<LOD	<LOD	<LOD	22.0	0.0
OBT 11		<LOD	<LOD	<LOD	<LOD	<LOD	9.5	0.0
OBT 12		<LOD	<LOD	<LOD	<LOD	<LOD	7.9	0.0
OBT 13	3 (n=5)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 14		<LOD	<LOD	<LOD	<LOD	<LOD	27.6	0.0
OBT 15		<LOD	<LOD	<LOD	<LOD	<LOD	4.3	0.0
OBT 16		<LOD	<LOD	<LOD	<LOD	<LOD	0.4	0.0
OBT 17		<LOD	<LOD	<LOD	<LOD	<LOD	15.8	0.0
OBT 18	4 (n=5)	<LOD	<LOD	<LOD	<LOD	<LOD	15.5	0.0
OBT 19		<LOD	<LOD	<LOD	<LOD	<LOD	11.0	0.0
OBT 20		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
OBT 21		<LOD	<LOD	<LOD	<LOD	<LOD	8.9	0.0
OBT 22		<LOD	<LOD	<LOD	<LOD	<LOD	7.5	0.0
CFT 1	5 (n=2)	<LOD	<LOD	<LOD	<LOD	2.4	<LOD	0.0
CFT 2		<LOD	<LOD	<LOD	<LOD	0.1	<LOD	0.0

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* Mean values of three independent determinations (n = 3) are presented as Mean (mg kg⁻¹).

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** Abbreviations employed: LOD (limit of detection), OBT (on-board brined tuna) and CFT (control frozen tuna).

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TABLE 54Biogenic amines assessment (mg kg⁻¹ muscle)* throughout various stages of skipjack tuna canning**

Con formato: Resaltar

Biogenic amine	Lot	Frozen brined tuna	Thawed tuna	Cooked solid tuna	Cooked grated tuna	Canned solid tuna	Canned grated tuna
Tryptamine	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Putrescine	1	<LOD ^b	<LOD ^b	<LOD ^b	1.7 ^a	<LOD ^b	<LOD ^b
	2	<LOD	<LOD	<LOD	1.2	<LOD	<LOD
	3	<LOQ	<LOD	<LOD	0.4	<LOD	<LOD
Cadaverine	1	<LOD ^b	1.1 ^b	2.9 ^b	9.6 ^{A,a}	1.8 ^b	4.2 ^{A,b}
	2	<LOD ^b	<LOD ^b	1.5 ^{ab}	2.5 ^{B,a}	0.9 ^{ab}	1.4 ^{B,ab}
	3	<LOD ^c	<LOD ^c	2.0 ^a	0.5 ^{B,bc}	0.9 ^b	<LOD ^{B,c}
Histamine	1	<LOD ^b	<LOD ^b	<LOD ^b	10.6 ^a	<LOD ^b	<LOD ^b
	2	<LOD	<LOD	4.9	<LOQ	<LOD	<LOD
	3	<LOD	<LOD	5.8	<LOD	<LOD	<LOD
Spermidine	1	4.2 ^b	13.0 ^b	3.8 ^{B,b}	66.7 ^{A,a}	20.6 ^b	92.8 ^a
	2	<LOD ^b	9.7 ^b	17.9 ^{AB,b}	60.8 ^{A,a}	2.2 ^b	70.6 ^a
	3	7.3 ^b	3.6 ^b	35.6 ^{A,b}	22.6 ^{B,b}	9.5 ^b	104.4 ^a
Spermine	1	4.2 ^b	7.2 ^a	<LOD ^{A,b}	<LOD ^b	<LOD ^b	<LOD ^b
	2	1.8	<LOD	1.8 ^B	<LOD	<LOD	<LOD
	3	<LOD	1.4	6.2 ^B	<LOD	<LOD	<LOD
Quality Index	1	0.0 ^b	0.1 ^b	2.2 ^a	0.4 ^{ab}	0.1 ^b	0.1 ^{A,b}
	2	0.0 ^b	0.0 ^b	0.2 ^{ab}	0.1 ^{ab}	0.6 ^a	0.0 ^{B,b}
	3	0.0	0.0	0.2	0.1	0.6	0.0 ^B

* Mean values of three independent determinations (n = 3) are presented. Within each column and for each amine, different capital letters (A-B) indicate significant differences (p<0.05); within each row and for each amine, different low-case letters (a-c) indicate significant differences (p<0.05). No letters are indicated when significant differences are not found (p > 0.05).

** Abbreviations employed: LOD (limit of detection) and LOQ (limit of quantification).

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TABLE 65

Con formato: Resaltar

Evolution of the pH value* in skipjack tuna muscle throughout various stages of skipjack tuna canning**

	Lot	Frozen brined tuna	Thawed tuna	Cooked solid tuna	Cooked grated tuna	Canned solid tuna	Canned grated tuna
pH	1	5.9	5.7	5.7 ^B	5.8	5.9	5.9
	2	5.9 ^a	5.7 ^b	5.9 ^{A,ab}	5.9 ^{ab}	5.9 ^{ab}	5.9 ^{ab}
	3	5.8 ^{a,b}	5.4 ^b	5.8 ^{AB,ab}	5.8 ^{ab}	5.9 ^{ab}	6.0 ^a

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* Mean values of three independent determinations (n = 3) are presented.
** Within each column, different capital letters (A-B) indicate significant differences (p<0.05) between lots; within each row, different low-case letters (a-b) indicate significant differences between stages. No letters are indicated when significant differences are not found (p > 0.05).

Fig. 1: Typical chromatograms of amines biogenic in standard solution (a), in control frozen tuna – CFT (b), on-board brined tuna – OBT (c). Tryptamine (1), Putrescine (2), Cadaverine (3), Histamine (4), Spermidine (5), IS (Internal Standard), Spermine (6) and unknown peaks (*). Separation conditions: mobile phases composed by deionized water and acetonitrile gradient elution, injection volume of 2 μ L, C18 column (100 mm x 3 mm x 3.5 μ m particle size) and security-guard column (50 mm x 3 mm x 3.5 μ m particle size), 40 $^{\circ}$ C, total run time of 12 min, wavelength 254 nm