

Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality

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Abstract

Biogenic amines are formed as a result of amino acid decarboxylation and are linked to food deterioration. Analysis of these metabolites may be of great importance to determine food quality. The aim of this study was to quantify the biogenic amines (putrescine and cadaverine), and evaluate the physicochemical parameters (pH, ammonia and total volatile bases) of rainbow trout meat (*Oncorhynchus mykiss*). Fifteen samples were packed in ice and transported in a Styrofoam container to the laboratory. Analyses were performed daily until the 15th day of storage. Biogenic amines concentrations and pH increased significantly throughout the storage period. No significant differences were observed in total volatile bases values over the time. Ammonia was detected after the 11th day of storage. Based on these results, cadaverine and putrescine may be used as a quality index of rainbow trout; however, total volatile bases may not be adequate parameter for this matrix.

Keywords: Physicochemical parameters; Biogenic amines; Quality index; Rainbow trout

Introduction

Fish and fishery products have played an important role in the human diet due to their high nutritional quality [1]; however, the chemical composition, high water activity, easily oxidized fat content, and pH close to neutral, accelerate its deterioration by promoting the development of the natural microbiota in this food matrix [2,3]. The deterioration occurs as a result of enzymatic and microbial activity, resulting in the production of different metabolites, which can lead to loss of product quality and can serve as quality indicators of the raw material [4,5].

The total volatile bases (monomethylamine, dimethylamine, trimethylamine, ammonia) are nitrogenous compounds originated from the degradation of some compounds in fish (amino acids and nucleotides) during the deterioration process [6,7]. The determination of total volatile bases (TVB) is one of the most widely used for assessing fish quality [6].

In freshwater fish, ammonia is considered a good indicator for evaluating the quality index, since it is the main compound of the group of substances evaluated in the analysis of TVB in these species [8]. Another indicator also widely used to evaluate the degradation process of fish is the hydrogen potential (pH). During the decaying process, there is the formation of alkaline compounds such as ammonia and amines, which accumulate in the muscle, increasing the muscle pH values [9].

Biogenic amines are formed as a result of amino acid decarboxylation, which is linked to the existence of spoilage bacteria in the food matrix [6,10,11]. Studies have reported that the biogenic amines, especially putrescine and cadaverine, can be considered good parameters for assessing the quality and the deterioration rate of various food matrices, including fish [3,11-15]. The development of analytical methods, faster than microbiological ones, for the identification and quantification of biogenic amines is very important to determine fish freshness [3,6]. Currently, chromatographic techniques offer a great advantage, since

they ensure accurate measurements and allow for the simultaneous analysis of several biogenic amines in fish and fishery products [16,17]. Among the chromatographic techniques, high-performance liquid chromatography (HPLC) is being widely used because of its sensitivity and reliability [18].

Due to the fact that there is limited scientific evidence demonstrating the effectiveness of biogenic amines as quality indicators of trout, and considering that rainbow trout (*Oncorhynchus mykiss*) is one of the main commercial aquaculture species produced and marketed worldwide [19], this study was conducted with the purpose of evaluating the use of biogenic amines and physicochemical parameters for quality assessment.

Materials and Methods

Sampling

Fifteen fresh rainbow trout (*Oncorhynchus mykiss*) specimens were obtained from the *Trutas da Serrinha* Company located in Itatiaia, a region of the Serra da Mantiqueira, in the state of Rio de Janeiro, Brazil. The samples were packed in ice ($0 \pm 1^\circ\text{C}$) and transported in a styrofoam container to the laboratory. The filet was obtained in sterile conditions, and all instruments used for filets dissection were previously sterilized.

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Received December 23, 2012; **Accepted** January 23, 2013; **Published** February 03, 2013

Citation: Rodrigues BL, Alvares TS, da Costa MP, Lopes Sampaio GS, de la Torre CAL, et al. (2013) Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and its Relationship with Physicochemical Parameters of Quality. J Aquac Res Development 4: 174 doi:10.4172/2155-9546.1000174

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The samples were analyzed daily until the 15th day of storage (Figure 1). All analyses were performed in duplicate.

Physicochemical analyses

pH, TVB and ammonia parameters were determined in order to evaluate the state of fish freshness during storage at $0 \pm 1^\circ\text{C}$. For the analysis of pH and TVB, potentiometric and microdiffusion methods were used, respectively Conte-Júnior et al. and Conway and Byrne [20,21]. The qualitative determination of ammonia was performed by using mercuric iodide, potassium iodide, sodium hydroxide solutions and water (Nessler reagent).

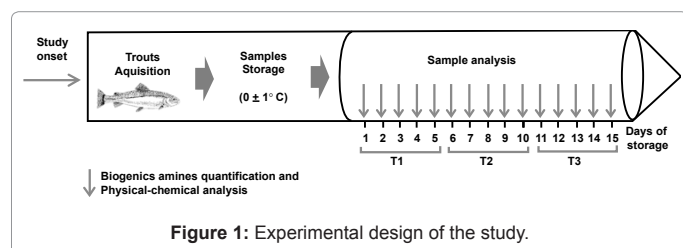
Biogenic amines quantification

The biogenic amines, putrescine and cadaverine, were assayed by High- Performance Liquid Chromatography (HPLC). Briefly, 5 mL of perchloric acid (5%) were added to 5 g of sample and kept 1 hour under refrigeration condition ($4 \pm 2^\circ\text{C}$) with periodic stirring. Subsequently the solution was centrifuged and filtered through Whatman filter paper No 1, followed by the addition of 2N sodium hydroxide to reach $\text{pH} > 6$. In the next step, the homogenized solution was kept in an ice bath for 20 minutes and filtered a second time, with the subsequent addition of 2N sodium hydroxide to reach $\text{pH} > 12$ [22]. Under these circumstances the solution was derivatized with the addition of 40 μL of benzoyl chloride, homogenized for 15 s and left to stand at room temperature for 20 min. Thereafter, 1 mL of diethyl ether was added and the supernatant was removed. The resulting sample was evaporated in a stream of nitrogen to be finally resuspended with 500 μL of mobile phase (acetonitrile: H_2O ; 42: 58; v: v) [23]. 20 μL of sample were injected into HPLC device coupled with UV detector; the flow rate was set at 1 mL min^{-1} . For the separation of the amine, a Teknokroma column, TR-016057 N26243 Tracer Extrasil ODS2 ($15 \times 0.46 \text{ cm}$, id. $5 \mu\text{m}$) and a Supelco precolumn, Ascentis C18 ($2 \times 0.40 \text{ cm}$, id. $5 \mu\text{m}$) were used.

Statistical analysis

The one-way ANOVA was performed to identify differences between biogenic amines (putrescine and cadaverine) and physicochemical parameters (TVB and pH) over the 15-day period of storage. When a significant *F* was found, additional post hoc tests with Bonferroni adjustment were performed. For the interpretation of result all the data obtained (from day 1 to day 15) were divided into three periods: Time 1 (T1)–analysis of the first five days of storage; Time 2 (T2)–analysis of the subsequent five days; Time 3 (T3)–analysis of the last five days of storage. Based on previous studies [11,14,24], no significant changes should occur in the biogenic amines concentrations and pH values at Time 1.

Statistical significance was set at the 0.05 level of confidence. All analyses were performed using a commercially available statistical package (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA).

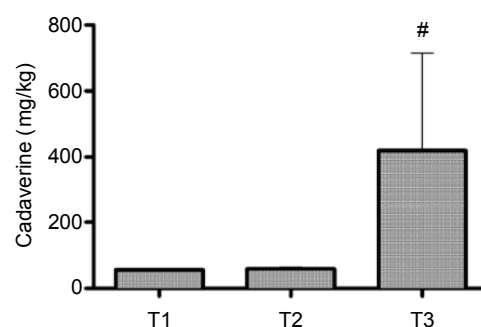


Results and Discussion

Recent studies do not report the use of quality physicochemical analyses that are considered simple and fast, and they have also not associated these analyses with biogenic amine concentrations to evaluate the quality of rainbow trout (*Oncorhynchus mykiss*). Therefore, the present study was designed to investigate the potential use of biogenic amines and physicochemical parameters—such as TVB, ammonia and pH—as a quality index for this species of fish. Overall, the major finding of the study was that the concentrations of the biogenic amine, putrescine and cadaverine, increased significantly—together with a significant increase in pH—over the 15-day storage period and the presence of ammonia was observed only after the 11th day of storage. No significant difference was observed in TVB values during the 15 days of storage.

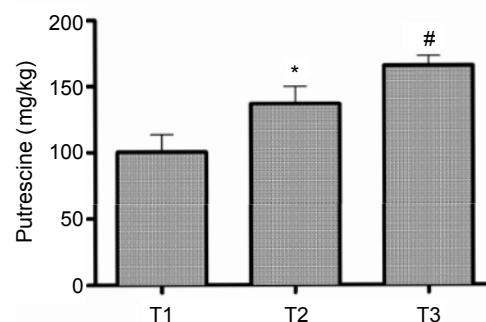
Changes in TVB and pH values over the period of the storage are shown in table 1. There was no significant difference in TVB values throughout the 15 days of storage. A significant increase in pH was observed in T3 as compared to T1. The presence of ammonia was observed only after the 11th day of storage.

The changes in cadaverine and putrescine concentrations over the days of storage are presented in figures 2 and 3, respectively. There was a



T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.
#Significantly different from T1 and T2.

Figure 2: Cadaverine concentrations (mg/kg) over the period of storage.



T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.
#Significantly different from T1 and T2.

*Significantly different from T1

Figure 3: Putrescine concentrations (mg/kg) over the period of storage.

significant increase in putrescine (T1: 100.17 ± 13.28 mg/kg; T2: 136.53 ± 13.28 mg/kg; T3: 165.62 ± 7.27 mg/kg) and cadaverine (T1: 56.49 ± 0 mg/kg; T2: 59.22 ± 4.97 mg/kg; T3: 419.75 ± 295.44 mg/kg) over the storage period.

According to EC Decision 95/149, there is no maximum limit for TVB; however, the limit was fixed to 25 mg N/100g in rainbow trout Gimenez et al. [25,26]. Although the present values at the end of storage period were below the limit suggested by Gimenez et al. the fish was in an evident state of sensorial deterioration, which had gone undetected by TVB analysis [26].

The compounds that form TVB are present in varying concentrations in muscle according to the kind of fish [7]. Unlike marine fish, freshwater fish generally have negligible values of trimethylamine oxide. Low levels of trimethylamine and the presence of ammonia are the main compounds that form part of TVB. Due to the low trimethylamine formation, the TVB concentration in freshwater fish species remains low during storage [8,27]. In addition, Morishita et al. reported that factors such as age, location and method of cultivation may influence the non-protein nitrogen compound content in the fish muscle, which may influence the TVB levels [28].

In the present study the TVB determination was not considered a good parameter for evaluating the quality index, since the values observed in this analysis did not even change at the end of storage period (Table 1) when the fish reached a state of deterioration (Figures 2 and 3). Other studies have confirmed the present results, demonstrating that TVB is not a good parameter for evaluating the quality index of freshwater fish [24,27]. Therefore, TVB may be considered an uncertain and unreliable decay index for freshwater fish.

Regarding ammonia, there was a correlation with the state of sensorial deterioration, since it was detected after the 11th day of storage in the samples. Ammonia is the main compound belonging to this set of volatile bases in freshwater fish species. This analysis is therefore considered satisfactory for evaluating the process of protein degradation; hence it may be used to determine the quality index on these fish species [8].

The pH values increased continuously over the storage period, reaching higher values in T3 (6.77 ± 0.13). This increase was due to the production of basic compounds formed during the autolytic changes [6]. According to Rodriguez et al. the accumulation of alkaline metabolites, such as amines, promotes an increase in muscle pH, indicating a deterioration process [9].

When analyzing the results of biogenic amines, the putrescine and cadaverine concentrations increased significantly throughout the storage period. The behavior of these amines in trout flesh was also observed by other researchers [11,15]. Studying the biochemical changes in rainbow trout stored for 12 days, Rodriguez et al. observed an increase of putrescine while cadaverine was detected only after 9 days of storage [11]. The authors suggest that the presence of cadaverine

may serve as an indicator of muscle change, which is caused by increased activity of microorganisms. Furthermore, putrescine may be an indicator of premature muscle autolytic degradation, since it is formed during the first days of storage.

According Dawood et al. the rapid formation of putrescine in fish is due to high enzymatic activity (due to the microflora contaminant) that promotes both the decarboxylation of glutamic acid and arginine and the synthesis of ornithine, which results in putrescine formation [12]. The authors suggest that putrescine and cadaverine may be reliable indicators of fish spoilage. Likewise, Rezaei et al. studying the presence of biogenic amines in rainbow trout stored for 18 days, observed an increase of amines during the storage period and suggested that monitoring the putrescine levels may serve as an index to evaluate the freshness of rainbow trout [15].

According to Gram and Dalgaard and Halász et al. the increase and the formation of these amines is related to the bacterial load present in meat [3,29]. Halász et al. observed that the bacteria of the family Enterobacteriaceae are usually implicated in the formation of cadaverine [29]. On the other hand, bacteria of the genus *Pseudomonas* spp. are responsible for the formation of putrescine.

Other previous studies [4,15,30-32] have demonstrated that the formation of biogenic amines depends on several factors that may alter the concentration of these amines in the food matrix. These factors include: aquaculture conditions, food, fish species, body composition, storage and processing conditions, autolytic interactions, availability of free amino acids, and the presence of decarboxylase-active microorganisms.

Based on the results of the present study, it appears that the biogenic amines, putrescine and cadaverine, may be considered suitable indicators of the degradation process of rainbow trout meat. Furthermore, the presence of ammonia and changes in pH may be regarded as quality parameters to evaluate this species. Finally, the total volatile bases (TVB) were not useful in assessing the deterioration level of the fish studied.

Acknowledgments

The authors are thankful for the financial support of the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), process numbers E-26/111.933/2011, E-26/110.460/2012 and E-26/103.003/2012. BL Rodrigues was supported by the National Council for Scientific and Technological Development (CNPq). *Trutas da Serrinha* is gratefully acknowledged for providing samples. The authors would like to thank Ricky Toledano for the English revision of the manuscript.

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	T1	T2	T3
TVB (mg N/100g)	9.30 ± 0.58	9.07 ± 1.19	9.07 ± 0.0
pH	6.47 ± 0.19	6.73 ± 0.09	6.77 ± 0.13*

T1=analysis of the first five days of storage; T2=analysis of the subsequent five days; T3=analysis of the last five days of storage.

*Significantly different from T1 (only pH; P<0.05).

Table 1: Mean and standard deviation values for total volatile basis (TVB) and hydrogen potential (pH) over the period of storage.

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