The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2024), 48(1), 62-77

ORIGINAL RESEARCH PAPER

EFFECT OF GUTTING ON MICROBIOLOGICAL, CHEMICAL AND SENSORY PROPERTIES OF URECHIS UNICINCTUS STORED IN ICE AND AT CHILL TEMPERATURE

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Received on 18 April 2024
Revised on 19 June 2024

Abstract

This study investigates the effect of gutting on quality characteristics of *Urechis unicinctus*, stored in ice and at chill temperature (4°C), in terms of sensory assessment, total volatile basic nitrogen, biogenic amines and microbial changes. The gutted and ungutted groups were kept in ice (T1-gutted, T2-ungutted) and at chill (4°C) temperature (T3–gutted, T4-ungutted). The study revealed that gutting worsened the sensory quality (as indicated by hardness, cohesiveness, springiness and chewiness) of all samples and that samples kept at 4°C scored lower than those kept in ice. Total Volatile Basic Nitrogen registered the highest values in gutted samples, exceeding the acceptable limit after 1 day for the T3, and 12 days for T1 samples, respectively. For ungutted samples the threshold value of 30 mg/L has been exceeded after 4 days for T4, and 14 days for T2 samples. In the present study, eight biogenic amines were analyzed; tryptamine and histamine were not detected all the time. The total concentration of biogenic amines in gutted *U. unicinctus* was significantly higher in comparison with ungutted groups starting from the 8th day, for samples stored in ice, and from the 3rd day for samples kept at 4°C ($p < 0.05$). However, the concentration of biogenic amines did not reach the allowable limit, even the samples were unacceptable due to high values of total viable count. Therefore, limits of biogenic amines available for fish are not suitable for *U. unicinctus.* Sensory scores were significantly correlated with Total Volatile Basic Nitrogen and Total Viable Count in all four groups ($p < 0.05$). Combined results of this study indicated that shelf life of T1, T2, T3 and T4 were 6, 7, 1 and 4 days, respectively. Gutting had a little effect on the storage of *U. unicinctus* stored in ice, but obviously shortened the shelf life of samples when stored at 4°C.

Keywords: *Urechis unicinctus*, gutting, shelf life, microbiological quality, chemical quality, sensory quality

https://doi.org/10.35219/foodtechnology.2024.1.04

Introduction

Urechis unicinctus, which belongs to the *Echiurioidea*, lives in marine sediments of the lower intertidal and subtidal zones in coastal mud or sandy areas, being mainly distributed around the coasts of China, Korea, Russia and Japan (Abe *et al.*, 2014; Zhang *et al.*, 2018). Fresh *U. unicinctus* is not just delicious, but also nutritious, it contains rich protein, various essential amino acids and a variety of trace elements in the body wall (Tan *et al.*, 2016). Due to its high nutrition, and similar shape, it is also called "naked sea cucumber" (Feng *et al.*, 2019). Major edible part of *U. unicinctus* is the body wall, which accounts for 32% of the whole-body weight, while the rest is represented by the gut (Meng *et al.*, 2008).

Compared to terrestrial meat, aquatic product is prone to corrupt because of high-level moisture, rich nutrient content, and high-activity enzyme in their body (Fan *et al.*, 2014). Generally speaking, quality degradation of the aquatic product during storage begins with the autolysis caused by endogenous enzymes, followed by microbial activity from gastrointestinal tract. Aquatic product gut is known to be a reservoir of strong digestive enzymes and bacteria (Haard *et al.*, 2008). Many spoilage bacteria in seafood were found to be abundant in the guts of various fish species (Shehata *et al.*, 2020). In this context, gutting may largely influence the microbiota of the flesh, and then the shelf life and spoilage patterns of the products (Zhuang *et al.*, 2021). Many studies have reported an extension of the shelf life of different aquatic products as a result of gutting (Karl & Meyer, 2007; Chytiri *et al.*, 2004). However, other studies have shown that gutting did not improve the quality or even decreased the shelf life of samples during chilled storage (Cao *et al.*, 2020; Viji *et al.*, 2015).

Marine invertebrate organisms such as sea cucumber are characterized by sedentary nature, filter feeding, permanent contact with sediment and thus specific and unspecific benthic bacterial community; hence, the risk of high gut bacterial load. In this case, gutting could increase the cross-contamination risk (Bogatyrenko & Buzoleva, 2016).

Various preservation techniques have been employed to improve the safety and prolong the shelf life of aquatic products, including chilling, icing, freezing, chemical preservation, salting or smoking (Sabu *et al.*, 2021; Özogul *et al.*, 2021). To keep taste quality, refrigeration technology is often used in the preservation of aquatic products.

At present, there are mainly two sale strategies for *U. unicinctus* in China, one is in live form and the other is in frozen form after gutting. In the former case, *U. unicinctus* are transported alive from farms to the market, and then temporarily kept in seawater for sale, this model is costly and affected by the transportation distance. In contrast, refrigerated sales are easier, requiring only a refrigerated cabinet or ice. Numerous studies have reported the quality changes of different aquatic products under refrigerated storage conditions; the most used indexes to predict the changes in freshness were sensory assessment (SA), total viable count (TVC), K value, total volatile basic nitrogen (TVB-N), 2-thiobarbituric acid (TBA) and biogenic amines

(BA) (Huang *et al.*, 2017; An *et al.*, 2015; Pawar *et al.*, 2020; Zhang *et al.*, 2015; Zhang *et al.*, 2011; Hernándeza *et al.*, 2009; Wu *et al.*, 2014; Biji *et al.*, 2016).

Hence, the selection of SA, TVC, TVB-N and BA was considered relevant for the objective of the present study. With the rising market demand, the efficient way to keep freshness of *U. unicinctus* has caused great concern among retailers and consumers (Zhang *et al.*, 2018). Gutting of *U. unicinctus* immediately after harvesting, has quantifiable benefits such a reduced transportation cost and lower environmental impact. However, the scientific information regarding the post-mortem changes and preservation aspects of gutted or ungutted *U. unicinctus* is missing. Due to its relatively new importance as a marketable product and the lack of information, a study of these changes during ice or refrigerated storage conditions is required.

Therefore, the main aim of the present study was to evaluate the effect of gutting on quality characteristics of *U. unicinctus* stored in ice and at refrigerated temperature (4°C) in terms of sensory assessment, TVB-N, BAs (biogenic amines) and microbial changes.

Materials and methods

Sampling

U. unicinctus was collected from a local aquatic market in the North Yantai region of China in May 2017, and was instantly delivered alive to the laboratory. The mean fresh mass and length of *U. unicinctus* were 62.2±4.8 g and 13.1±0.6 cm, respectively. Raw materials were washed with Milli-Q water and divided into four equal batches at random. The gutted and ungutted groups were kept in ice (T1, T2) and at 4°C (T3, T4). The gutted groups were treated as follows. First, both ends of the body were cut, then squeezed out the viscera, after washing, the body wall was left to drain. All samples were packed individually in polyvinyl chloride bags, T1 and T2 were covered with flake ice at *U. unicinctus*/ice (1:2 w/w) and all samples were stored in the refrigerator with the temperature controlled at 4°C. Ice was added as needed during storage. Three packaged samples (n=3) of each group were taken for random analysis.

Sensory Assessment (SA)

SA was performed following the procedure recommended by Ojagh. *et al.* (2010) with some modifications according to the characteristic of *U. unicinctus*. The sensory characteristics were evaluated by a well-trained panel of nine members (five females and four males, 20 to 35 years old) from the laboratory staff employed to evaluate the four groups of samples. Each assessor was requested to score all samples for the odor, appearance, elasticity, and morphology of *U. unicinctus* raw muscle. A 5-point grading system was adopted for each attribute, in which a score of 4.0–5.0 indicated excellent quality (perfect condition), 3.0–4.0 indicated high quality (slight loss of excellent characteristics), 2.0–3.0 indicated good quality (some deterioration, but fit for sale), 1.0–2.0 indicated acceptable quality, and less than 1.0 indicated unacceptable quality (Table 1). The total scores of each sample were estimated from

the sum of the average scores obtained by each assessor according to each of the four attributes.

Table 1. Descriptive terms related to sensory assessment for *U. unicinctus*

Scores	Appearance	Odor	Morphology	Elasticity
4.0-5.0	Fresh, shiny	Inherent fragrance, fresh	Very dense	Very flexible
3.0-4.0	Slightly darker, slight loss of shiny	Inherent fragrance, slight loss of fresh	Dense	Flexible
2.0-3.0	Slight discoloration, dull shiny	Slight inherent fragrance	Slightly dense	Slightly flexible
1.0-2.0	Obvious discoloration, dim	No inherent fragrance, slightly odorous	No dense	Little flexible
<1.0	Intensity discoloration, no shine	Intensity fishy odor	No dense，part loose	No elasticity

Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was determined by microtitration method (Song *et al.*, 2011) and expressed as mg/100 g flesh. The method was based on water vapor distillation and extraction of volatile base. The distillate was titrated with standard hydrochloric acid.

Total Viable Counts (TVC)

Total viable counts (TVC) were determined following the previous used method (Hu *et al.*, 2013) with some modifications. Five grams of *U. unicinctus* were aseptically weighed and homogenized in a solution containing 45 mL of sterile 0.9% physiological saline for 60 s. The homogenized samples were serially 10-fold diluted using sterile 0.9% physiological saline. Bacteria counts were determined on plate count agar and the inoculated plates were incubated for 2 days at 30℃. Data was expressed as logarithms of the number of colony forming units (log10 CFU/g). Microbiological analysis was performed in duplicate.

Biogenic amines (BAs)

BAs extraction was performed based on the procedure developed by Shi *et al.* (2012). *U. unicinctus* (5g) was homogenized with 10 mL 0.6 M cold perchloric acid (PCA) solution for 60 s. The homogenate was centrifuged for 15 min at 10,000 rpm. This operation was repeated two times. The supernatants obtained twice were merged and adjusted to 25 mL with 0.6 M PCA. A volume of 0.4 mL of the extracted sample was mixed with 0.12 mL of saturated $NaHCO_3$ and 0.08 mL of 2 M NaOH. In the solution, 0.8 mL of 10 mg/mL dansyl chloride solution prepared in acetone was added. Then, the reaction mixture was incubated for 45 min at 40℃ in darkness. To stop the reaction 40 µL ammonia (25%) was added. The mixture was adjusted to 2 mL with acetonitrile after 30 min, then filtered through 0.22 µm membrane prior to HPLC analysis. The quantification of BAs was conducted using LC-10AT HPLC unit (Shimadzu technology Inc. Kyoto, Japan) consisting of LC-10A(V) detector.

BAs were separated using a cosmosil 5C18-PAQ column (4.6×250 mm). A gradient elution of 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B) were used as mobile phases. Elution procedure was performed using the following gradient: 0 min, 50% B; 25 min, 90% B; 35 min, 90% B; 45 min, 50% B. Sample (20 µL) was injected at a flow rate of 0.8 mL/min and temperature of column was set at 30°C. The detecting wavelength was 254 nm. A standard stock solution containing 1 mg/ml of each biogenic amine including cadaverine di-hydrochloride, putrescine dihydrochloride, tryptamine hydrochloride, histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride, spermine tetrahydrochloride and spermidine trihydrochloride was prepared in 50 ml 0.1 M HCl. The standard stock solution was serially diluted with 0.1 M HCl to obtain different working solutions to make the standard curve.

Statistical Analysis

All the experiments were performed in triplicate (except TVC, which was carried out in duplicate). Data distribution was evaluated by the Kolmogorov-Smirnov and Shapiro-Wilk tests, while Levene's test assessed the homogeneity of variances. ANOVA and Tukey's tests evaluated the variance in the dataset. The significance for all tests was $p < 0.05$, and SPSS version 20.0 (IBM, Chicago, IL, USA) was used for data analysis. Student's t-test was used to find the significant differences among BA concentrations from gutted and ungutted samples from each thermal regime during storage. Pearson's correlation analysis was performed to determine the relationship among physical, microbial and chemical quality.

Results and discussion

Sensory analysis

The changes of sensory scores are presented in Figure 1. Initial sensory scores of *U. unicinctus* were about 20, which showed that the four groups of samples were all perfectly fresh. There were no significant differences ($p > 0.05$) among T1, T2, T3 and T4 initial samples. With the extension of storage time, sensory scores of each group obviously decreased. Scores of T2 were higher than the other three groups on every day except the first storage day. T3 and T4 had a sharp decline compared with T1 and T2 during the storage period. Over the entire storage period, the scores of ungutted samples were higher than the gutted ones under the same storage conditions. Total sensory score of 4.0 was used as the sensory unacceptability point. The acceptable shelf life was found to be 3 days for T3 (score 3.62 on 4th), 4 days for T4 (score 3.47 on 5th), 6 days for T1 (score 3.9 on 7th) and 7 days for T2 (score 4.1 on 7th). Besides, the initial color of all samples was very bright reddish-brown appearance, while as storage time went on, the color gradually faded to gray. The development of faded color, unpleasant odor, slimy and inelastic texture directly characterized the corruption progress of aquatic products.

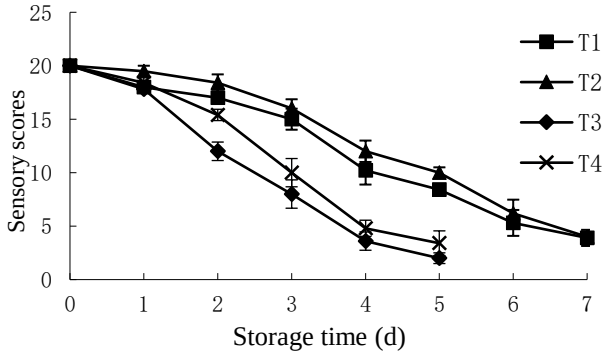

Figure 1. Changes in sensory scores of *U. unicinctus* during storage in ice for 7 days and at 4℃ for 5 days. T1: gutted, 0℃; T2: ungutted, 0℃; T3: gutted, 4℃; T4: ungutted, 4℃.

In a sensory assessment trial, Karl and Meyer (2007) found that haddock, stored in ice, remained within acceptable limits for 15 days when gutted, while the acceptance for saleability of ungutted groups was 11 days. In this case, the difference in shelf life of haddock was mainly attributed to the high enzyme activity in the gut which hastened the deterioration of belly flaps. These findings weren't in accordance with our results, which indicated that integrity of raw material had an obvious effect on the freshness of *U. unicinctus* stored in ice and at 4 ℃. Gutting makes *U. unicinctus* lose its original shape, then the soft body wall sticks together, which reduces the sample's sensory scores. Similar results for assessments of shelf life of whole ungutted and gutted sea bass stored in ice have also been reported (Kilinc *et al.*, 2006).

Total Volatile Basic Nitrogen (TVB-N)

Proteins in seafood are decomposed by endogenous enzymes and spoilage bacteria, resulting in nitrogen-containing volatile substances, which collectively are known as TVB-N (Lu *et al.*, 2009). Beside toxicity, these compounds are responsible for color and flavor changes that limits the meat products acceptability (Cao *et al.*, 2013). Therefore, TVB-N value is often used as a chemical indicator to evaluate the freshness of seafood samples during storage. Changes in the values of TVB-N for T1, T2, T3 and T4 are summarized in Figure 2. The initial TVB-N value was 10.78 mg/100 g for all the samples. Compared with the recommended acceptable limit of TVB-N (30 mg/100 g) (Gökodlu *et al.*, 1998), *U. unicinctus* kept acceptable quality up to 12, 14, 1 and 4 days for T1, T2, T3 and T4 groups, respectively.

TVB-N values of T3 group, with a sharp increase after first day, were significantly higher than the other three groups ($p < 0.05$). During earlier storage, there was no significant increase in TVB-N concentration from 0 day to the 12th day ($p > 0.05$) for T2 group; however, after 12 days, the concentration began to increase rapidly with storage time. Similar results, where TVB-N value increased quickly at the end of storage, were also obtained in other studies (Zhang *et al.*, 2015; Biji *et al.*, 2016; Liu *et al.*, 2017). Other authors (Viji *et al.*, 2015) reported no significant increase in TVB-N value in the first 5 days, for both gutted and ungutted sutchi catfish stored in

ice. Nevertheless, after 5 days, the TVB-N values in gutted catfish was significantly lower ($p < 0.05$) than in ungutted sample, which was not the case of the present trial. In the present study, the TVB-N values were higher in gutted *U. unicinctus* compared with the whole ungutted samples over the entire storage period. This observation agrees with the findings reported for ungutted and gutted sea bass during ice storage (Papadopoulos *et al.*, 2003).

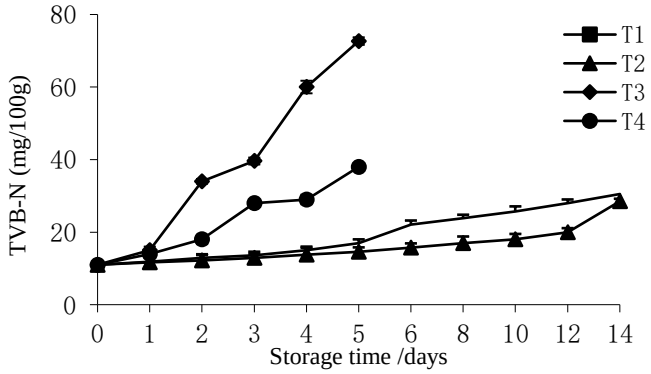

Figure 2. Changes in TVB-N of *U. unicinctus* during storage in ice for 14 days and at 4°C for 5 days. T1: gutted, 0°C; T2: ungutted, 0°C; T3: gutted, 4°C; T4: ungutted, 4°C

TVB-N index is a commonly used indicator for the assessment of spoilage degree in seafood. Compared with fish, TVB-N values in *U. unicinctus* changed rapidly. Fan *et al.*, 2014, reported that black carp fillet samples, stored at 4 °C, maintained good quality up to 8 days within the highest acceptable level of TVB-N. In the present study, when *U. unicinctus* was unacceptable according to sensory analysis, TVB-N value was still below the commonly acceptance limit established for most fish species (30 mg/100 g): 16.8 mg/100 g for T2 group and 26.4 mg/100 g for T1. Therefore, it seems that TVB-N value cannot be the major index of spoilage of *U. unicinctus* stored in ice, which is consistent with other reports (Özogul & Özogul, 2004; Özogul *et al.*, 2006).

Biogenic amines (BAs)

Concentrations of BAs in *U. unicinctus* during ice and chilled storage are presented in Table 2 (a, b). Eight biogenic amines were analyzed, among which tryptamine and histamine were not detectable in our samples. Although histamine is the most important biogenic amine from a toxicological point of view, in the present study, it was not present in any of the tested samples. This result agreed with other studies on the spoilage of rainbow trout (*Oncorhynchus mykiss*) during ice storage where histamine (Özogul & Özogul, 2004) and tyramine (Rezaei *et al.*, 2007) were not found. Except as a physiological component of live aquatic products, the formation of BAs is mainly related to the microbial growth and spoilage (Křížek *et al.*, 2011). Production of BAs depends upon various conditions such as species, body composition, storage and processing methods, the numbers of decarboxylase-active microorganisms and the concentration of specific free amino acids (Křížek *et al.*, 2011).

In the present study, the concentration of putrescine increased significantly from the 3rd day for T3 and from the 8th day for T1 and T2 ($p < 0.05$). Cadaverine was not detected on the first testing day, but the values increased continuously and significantly in all four groups during the whole storage period ($p < 0.05$). Because of the rapid increase of cadaverine and putrescine, these two amines became the dominant biogenic amine in all variants during later storage. However, for both amines, the concentrations were higher in gutted *U. unicinctus* samples, for both storage conditions. Likewise, other authors (Özogul & Özkan, 2013) concluded that putrescine and cadaverine were the most accumulated biogenic amines in sea bream (*Sparus aurata*) during ice storage. The corruption process of various seafood could cause the increase of cadaverine and putrescine. Therefore, these two biogenic amines could be applied as indicators to evaluate the freshness or safety of seafood. For this purpose, some authors (Yamanaka *et al.*, 1989) proposed an upper acceptable limit for cadaverine as 100 mg/kg. In our study, the cadaverine contents of the four groups did not exceed the recommended limit. On the other hand, for putrescine, concentrations below 10 mg/kg were correlated with good quality of carp flesh, concentrations between 10-20 mg/kg were associated with acceptable quality while values above 20 mg/kg were attributed to poor flesh carp quality (Honda *et al.*, 2002). Nevertheless, for other aquatic species different thresholds for human consumption have been proposed for putrescine: for cephalopods 17 mg/kg (Altissimi *et al.*, 2017), for shucked mussel (*Mytilus galloprovincialis*) 60 mg/kg (Erkan *et al.*, 2005), for shrimps and Norway lobster 3 and < 7 mg/kg. From this work it is possible to set limits of freshness acceptability of 30 mg/kg of putrescine according to TVB-N for *U. unicinctus*. This limit was exceeded after 8 days in T1 and after 3, respectively 5 days, for T3 and T4 (Table 2 a, b).

It was reported that the occurrence of putrescine and cadaverine was generally related to the activity of ornithine and lysine-decarboxylase of Enterobacteriaceae, respectively (Bover-Cid *et al.*, 2001).

Values of phenylethylamine were in a fluctuant downward trend for all groups, while spermine concentrations of four groups increased with fluctuation during storage. There were no significant differences in spermidine contents both for T3 and T4 with the storage duration ($p > 0.05$). As for the change of tyramine concentrations, T1 and T2 showed a trend of decreased fluctuation, and T3 and T4 also had a slight variation during the storage. However, the maximal tyramine concentrations were below the dietary acceptable limit of 100 mg/kg (Brink *et al.*, 1990). Decrease of biogenic amine concentrations could be attributed to the amine's oxidization. Spermine and spermidine are naturally present in food, so their production is not associated with microbial spoilage.

The total concentration of BAs in gutted *U. unicinctus* was significantly higher than in ungutted treatment from the 8th day stored in ice and the 3rd day stored at 4℃ ($p < 0.05$). Previous studies have shown that BAs formation in food is a result of microbial action during storage. That means the process of viscera removal in *U. unicinctus* not only failed to reduce the content of microorganisms but also increased the risk of contamination.

Table 2. Biogenic amines (BA) concentration (mg/kg) in *U. unicinctus* during storage in ice for 12 days (a) and 4˚C for 5 days (b)

BA (a)	Treatment	Storage time (days)					
		0	**2**	**5**	**8**	**10**	**12**
Phe.	T1	11.03±1.1^{a}	0.67±0.21^{c}	0.41±0.15^{c}	3.46±0.49^{b}	1.01±0.22^{c}	1.15±0.29^{c}
	T2	11.03±1.1^{a}	1.54±0.45^{b}	ND	1.43±0.23^{b}	1.41±0.15^{b}	1.3±0.36^{b}
Put.	T1	12.58±1.62^{b}	14.66±2.33^{b}	18.43±2.06^{b}	39.92±2.89^{a}	43.01±3.52^{a}	56.03±2.33^{a}
	T2	12.58±1.62^{b}	12.68±3.64^{b}	13.05±1.47^{b}	15.80±1.39^{ab}	17.87±2.09^{ab}	21.91±2.41^{a}
Cad.	T1	ND	7.89±1.06^{e}	19.55±1.44^{d}	34.78±2.45^{c}	65.73±4.05^{b}	80.73±4.56^{a}
	T2		5.65±1.03^{e}	11.85±1.72^{d}	28.05±1.88^{c}	34.70±2.56^{b}	41.39±2.58^{a}
Tym.	T1	23.80±3.78^{a}	3.30±0.38^{c}	12.75±3.18^{b}	6.05±0.68^{c}	9.25±1.14^{bc}	18.18±1.54^{ab}
	T2	23.80±3.78^{a}	20.35±1.99^{a}	14.47±1.21^{ab}	11.70±1.24^{b}	10.23±0.99^{b}	8.74±0.76^{b}
Spmd.	T1	6.53±1.05^{ab}	4.29±0.52^{b}	4.92±1.22^{ab}	7.79±0.83^{a}	7.55±0.6^{a}	6.87±1.66^{ab}
	T2	6.53±1.05^{a}	4.03±1.02^{b}	6.01±1.01^{ab}	5.60±0.77^{ab}	4.62±0.12^{ab}	4.73±0.43^{ab}
Spm.	T1	1.28±0.13^{c}	15.79±1.069^{b}	16.86±1.36^{b}	16.90±1.85^{b}	21.51±1.03^{a}	16.28±1.85^{b}
	T2	1.28±0.13^{c}	10.33±1.02^{b}	20.72±1.59^{a}	12.77±1.14^{b}	13.12±1.24^{b}	22.62±1.21^{a}
Total	T1	55.21±7.68^{e}	46.57±6.19^{e}	72.92±9.41^{d}	108.9±9.19^{c*}	148.07±10.56^{b*}	179.25±12.23^{a*}
	T2	55.21±7.68^{cd}	54.58±9.15^{d}	66.09±7^{c}	75.35±6.65^{b*}	81.96±7.15^{b*}	100.72±7.75^{a*}

BA (b)	Treatment	Storage time (days)					
		0	1	2	3	4	5
Phe.	T3	11.03±1.1^{a}	9.79±1.22^{a}	ND	6.06±1.07^{b}	4.57±1.25^{b}	8.59±1.43^{ab}
	T4	11.03±1.1^{a}	10.23±1.65^{b}	8.15±1.73^{b}	9.14±1.17^{b}	10.38±1.3^{b}	7.18±1.27^{b}
Put.	T3	12.58±1.62^{d}	15.98±1.72^{d}	19.50±1.59^{d}	45.39±3.11^{c}	63.61±3.04^{b}	77.93±4.13^{a}
	T4	12.58±1.62^{b}	13.13±2.59^{b}	14.15±1.56^{b}	24.49±2.83^{a}	23.43±4.08^{a}	30.50±3.5^{a}
Cad.	T3	ND	3.78±0.75^{e}	9.22±1.21^{d}	53.69±7.45^{c}	77.27±7.76^{b}	98.09±8.23^{a}
	T4		6.48±1.12^{d}	6.31±0.99^{d}	35.12±4.69^{c}	47.64±5.12^{b}	60.96±6.42^{a}
Tym.	T3	23.80±3.78^{b}	21.03±3.45^{b}	26.39±5.86^{ab}	30.94±3.45^{a}	22.17±3.25^{b}	26.98±3.12^{ab}
	T4	23.80±3.78^{a}	22.52±2.36^{a}	24.06±4.65^{a}	23.29±3.56^{a}	23.00±4.23^{a}	20.27±2.68^{a}
Spmd.	T3	6.53±1.05^{a}	5.71±0.88^{a}	5.38±0.99^{a}	6.63±0.79^{a}	6.39±1.32^{a}	5.34±1.04^{a}
	T4	6.53±1.05^{a}	6.06±0.98^{a}	7.03±0.79^{a}	7.19±0.88^{a}	6.35±1.12^{a}	6.29±1.64^{a}
Spm.	T3	1.28±0.13^{c}	14.63±0.98^{b}	15.04±1.46^{b}	13.55±1.33^{b}	14.05±1.45^{b}	18.30±1.62^{a}
	T4	1.28±0.13^{c}	15.90±1.56^{b}	20.21±2.35^{a}	20.78±1.98^{a}	18.43±1.98^{ab}	16.25±2.21^{b}
Total	T3	55.21±7.68^{e}	70.93±9.0^{d}	75.54±11.1^{d}	156.25±17.2^{c*}	188.04±18.07^{b*}	235.23±19.57^{a*}
	T4	55.21±7.68^{d}	74.4±10.26^{c}	80.22±12.07^{c}	116±15.11^{b*}	129.24±17.8^{ab*}	141.45±17.72^{a*}

Legend: Phe.- Phenylethylamine; Put. – Putrescine; Cad.- Cadaverine; Tym.- Tyramine; Spmd.- Spermidine; Spm.- Spermine; ND, not detected.

Note: Results are presented as mean ± standard deviation. Same superscript lowercase letters in a row indicate no significant differences ($p > 0.05$) among storage days. * Symbols in a column reflect significantly different means between sample groups from the same storage conditions ($p < 0.05$).

Total BA concentrations in all samples of the four groups were lower than the allowable limit (1000 mg/kg) in foods (Silla Santos, 1996), even though the samples were unacceptable at the later storage according to TVC. This is strong evidence that the allowable limit of BAs in the literature is not suitable for *U. unicinctus.*

Total viable count (TVC)

Microbial deterioration is one of the most common causes of fish spoilage, therefore TVC has been used in the seafood quality standards in many countries. Variations in TVC values during storage are presented in Figure 3.

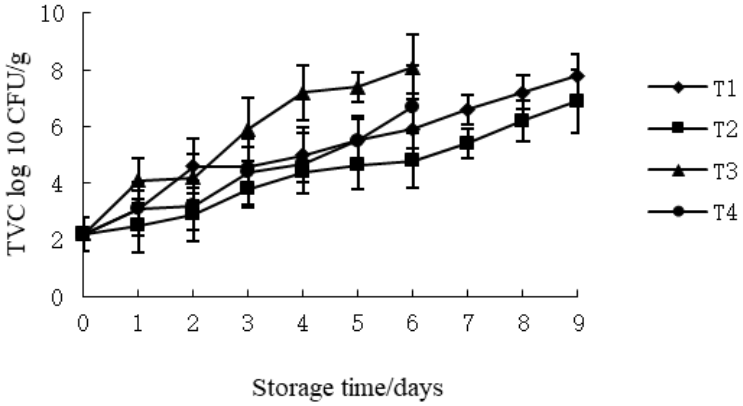

Figure 3. Changes in microbial characteristics (log 10 CFU/g) of *U. unicinctus* during storage in ice for 9 days and at 4°C for 6 days. T1: gutted, 0°C; T2: ungutted, 0°C; T3: gutted, 4°C; T4: ungutted, 4°C

According to previously published data (Hernàndeza *et al.*, 2009), the upper acceptability limit for seafood is 7 log10 CFU/g, which indicates the high quality of raw *U. unicinctus* materials used in this study (2.25 log10 CFU/g). Other publications reported a similar original number of bacteria in the bighead carp (Hong *et al.*, 2013). In the present experiment, TVC values increased over time in all experimental variants. A similar pattern, of continuous increase of TVC in seafood during storage, has been previously reported (Parlapani *et al.*, 2018). However, for T3 samples, TVC registered the highest numbers among the four groups, the mean values being significantly higher than T2 ($p < 0.05$). Compared with the upper acceptability limit, it showed that *U. unicinctus* maintained edible quality up to 7, 9, 3 and 6 days for T1, T2, T3 and T4, respectively. Bacteria grew more quickly in gutted *U. unicinctus* than in ungutted samples during the whole storage. Evisceration resulted in tissue damage, which provided a good medium for microbial growth. Similar reports (Papadopoulos *et al.*, 2003) found that bacterial populations of gutted sea bass were higher than those obtained for whole ungutted fish samples throughout the entire period of storage in ice. TVC concentrations were 6.6, 5.4, 5.9 and 5.5 log10CFU/g for T1, T2, T3 and T4 when *U. unicinctus* reached the acceptability limit in terms of sensory analysis.

Relationship among biogenic amines and quality attributes

The correlation matrix of biogenic amines to sensory scores, TVB-N and TVC during conditions of chilled and iced storage of *U. unicinctus* are presented in Tables 3 and 4.

Table 3. Correlation among biogenic amines, TVB-N, TVC and sensory scores of *U. unicinctus* during ice storage

Processing		Phe.	Put.	Tym.	Spmd.	Spm.	Cad.	S. s.	TVBN (mg/100 g)
T1	Put.	-0.400							
	Tym.	0.652	-0.035						
	Spmd.	0.281	0.643	0.256					
	Spm.	-0.926**	0.528	-0.722	0.026				
	Cad.	-0.502	0.959**	-0.007	0.533	0.601			
	S.s.	0.532	-0.947**	0.045	-0.490	-0.625	-0.997**		
	TVBN (mg/100g)	-0.544	0.959**	-0.234	0.620	0.705	0.925**	-0.913*	
	TVC (log10 CFU/g)	-0.731	0.820*	-0.192	0.323	0.751	0.840*	-0.826*	0.889*
T2	Put.	-0.375							
	Tym.	0.795	-0.783						
	Spmd.	0.600	-0.287	0.305					
	Spm.	-0.829*	0.561	-0.807	-0.348				
	Cad.	-0.608	0.927**	-0.942**	-0.309	0.645			
	S. s.	-0.688	0.444	0.688	0.106	0.721	-0.857*		
	TVBN (mg/100 g)	0.671	-0.442	0.673	-0.136	-0.738	0.845*	-0.998**	
	TVC (log10 CFU/g)	0.577	-0.287	-0.555	-0.273	-0.588	0.738	-0.976**	0.975**

Legend: Phe.- Phenylethylamine; Put. – Putrescine; Cad.- Cadaverine; Tym.- Tyramine; Spmd.- Spermidine; Spm.- Spermine; S.s.- Sensory score; * Statistical differences ($p < 0.05$).**Statistical differences ($p < 0.01$).

Table 4. Correlation among biogenic amines, TVB-N, TVC and Sensory scores of *U. unicinctus* during refrigerated storage

Processing		Phe.	Put.	Tym.	Spmd.	Spm.	Cad.	S. s	TVBN (mg/100g)
T3	Put.	-0.543							
	Tym.	-0.533	0.998**						
	Spmd.	-0.526	0.275	0.315					
	Spm.	0.760	-0.327	-0.283	0.122				
	Cad.	-0.746	0.555	0.548	0.175	-0.673			
	S. s	0.654	-0953**	-0.671	-0.378	0.427	-0.953**		
	TVBN (mg/100 g)	-0.686	0.967**	0.643	0.293	-0.519	0.958**	-0.980**	
	TVC (log10 CFU/g)	-0.543	0.928**	0.752	0.274	-0.335	0.933**	-0.954**	0.912*
T4	Put.	0.017							
	Tym.	-0.013	0.975**						
	Spmd.	-0.306	-0.824*	-0.803					
	Spm.	-0.314	-0.382	-0.529	0.620				
	Cad.	-0.656	0.400	0.427	-0.217	-0.200			
	S. s.	0.189	-0.942**	-0.521	0.738	0.498	-0.983**		
	TVBN (mg/100 g)	-0.128	0.982**	0.520	-0.814*	-0.423	0.978**	-0.976**	
	TVC (log10 CFU/g)	-0.072	0.937**	0.508	-0.846*	-0.620	0.978**	-0.978**	0.971**

Legend: Phe.- Phenylethylamine; Put. – Putrescine; Cad.- Cadaverine; Tym.- Tyramine; Spmd.- Spermidine; Spm.- Spermine; S.s.- Sensory score; *Statistical differences ($p < 0.05$).**Statistical differences ($p < 0.01$).

Putrescine and cadaverine showed significant ($p < 0.01$) correlations with sensory scores, TVB-N and TVC for all four groups except for T2. As it was previously stated, cadaverine and putrescine became the dominant biogenic amine during later storage. These two biogenic amines increased rapidly with the extension of storage time, and the trend was consistent with changes in sensory and microbiological indicators. Laly *et al.* (2020) has reported that TVB-N showed a significant ($p < 0.01$) correlation with putrescine and cadaverine in three spotted crabs during conditions of refrigerated and iced storage. Hu *et al.* (2012) stated that strong positive correlations ($R^2 > 0.9$) existed between TVC and the concentration of putrescine and cadaverine in blue scad during storage at 4℃ and 25℃. Since the changes of these two biogenic amines are closely related to the quality of *U. unicinctus* during storage, they could be used as freshness evaluation indicators.

Sensory scores were significantly correlated with TVB-N, TVC with high Pearson's correlations ($p < 0.05$) during both storage conditions. Similar reports showed that the drip loss, a physical index which indicated the texture loss of fish, was significantly correlated with TVC and TVB-N (Hong *et al.*, 2013).

The deterioration and quality loss of aquatic products are mainly caused by the action of enzymes and microbes (Arashisar *et al.*, 2004). However, the spoilage process includes not only the degradation of proteins, resulting in elevated TVB-N and BAs, but also the hydrolysis and oxidation of lipids, and the cleavage of triglycerides by triglyceride lipases or excretion by certain microorganisms, resulting in increased free fatty acid and peroxide values (Viji *et al.*, 2015). Theoretically, the quantity of digestive enzymes and microbes can be greatly reduced by removing the internal organs, then it would lead to lower tissue spoilage in gutted aquatic products compared with ungutted samples. The viscera of *U. unicinctus* accounts for 68% of total body weight (Meng *et al.*, 2008). Therefore, removing viscera could not only save energy but also prolong the shelf life of *U. unicinctus* during the transport and sale process, theoretically. Other studies (Karl & Meyer, 2007) have demonstrated that immediate removing viscera after catch could prolong the shelf-life of haddock, plaice and saithe stored in ice. Gutting increased the shelf life to 4 days in both Tilapia and Nile perch stored in ice (Lokuruka *et al.*, 2012). However, in this study, all the detected indexes indicated that gutting shortened the shelf life of *U. unicinctus* under the same storage condition. This may be attributed to cross-contamination of samples caused by the gutting procedure, and on the other hand, direct exposure of the cutting surface to air rendering the lipid more susceptible to oxidation (Erkan & Ozden, 2006). There are variations among the reports about the effect of gutting on the shelf life of aquatic products in the literature. One of these studies (Chytiri *et al.*, 2004) demonstrated that the shelf life of filleted trout stored in ice was 10-12 days; for the ungutted samples, however, the shelf-life was extended to 15-16 days during ice storage. On the other hand, for the sea bream, gutting had no effect on the shelf life during chill storage; though gutting decreased the bacterial load in the early days of storage, no differences were found between the gutted and ungutted samples at the point of sensory rejection (Ghaly *et al.*, 2010). For sea bass, the shelf life of whole ungutted and gutted samples was 13 and 8 days respectively during ice storage

(Papadopoulus *et al.*, 2003). Doubtless, the process behind spoilage of aquatic products is highly complex depending on a series of factors such as: the species, the lipid content, the rearing environment, the harvesting method; whether the gutting of fish could be advantageous or not remain to be determined specifically Tejada & Huidobro, 2002).

Conclusions

Gutting could reduce the amounts of microorganisms and enzymes triggering spoilage, and from a commercial point of view, gutting decreases environmental contamination and reduces transportation costs. Nevertheless, gutting may also lead to problems such as cross-contamination, which may result in an increased spoilage rate. The results of the present study indicated that the sensory analysis was the key index when stored in ice, and gutting had a little effect on the shelf life, so it was not necessary to gut when stored in ice. However, at chill temperature, gutting resulted in a rapid increase of the TVB-N values and the microbial load, so it was concluded that gutting was not indicated for *U. unicinctus* stored at 4°C. The shelf life of *U. unicinctus* kept in ice was 6 days for gutted and 7 days for the ungutted samples, while for the refrigerated conditions the shelf life was 1 day for gutted and 4 days for the whole ungutted samples.

In the present study, the lipid damage evaluation was not approached and therefore further investigations on this subject will be necessary. Likewise, for a deeper knowledge, studies on the spoilage mechanism and dominant spoilage bacteria during the storage are required in order to develop corresponding preservation measures to extend the shelf life of *U. unicinctus*.

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