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Quality assessment of wild European eel (*Anguilla anguilla*) stored in ice

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Abstract

The quality assessment of wild European eel (*Anguilla anguilla*) stored in ice and in boxes without ice (3 ± 1 °C) was investigated by the sensory analysis, levels of nucleotide breakdown products and biogenic amines for up to 19 days. Sensory analysis was assessed using the Tasmanian Food Research Unit Scheme. *K* and related values (K_i, *G*, *P*, *H* and F_r) were used as freshness indicators. Linear regressions (r^2) obtained from *K*, K_i, *G*, *P*, *H* and F_r were 0.95, 0.96, 0.83, 0.96, 0.99 and 0.96, respectively, for eel stored in ice whereas, for eel kept in boxes without ice, the values were 0.86, 0.86, 0.96, 0.91, 0.98 and 0.86, respectively. When eel stored in ice and in boxes without ice were considered at the limit of acceptability by assessors at ~12–14 days and ~5–7 days, respectively, the average *K*, K_i and *P* values were ~70–85%, *H* values were ~60% and F_r values were ~10% for both storage conditions. The level of histamine exceeded the legal limit (5 mg/100 g fish) in eel stored without ice after 6–7 days and, in ice, after 13–14 days of storage, at which time eels were rejected by the sensory panel. The concentrations of biogenic amines were higher in eel stored in boxes without ice than in eel kept in ice. The levels of histamine in the muscle of eel kept in boxes without ice and in ice increased to the maximum levels of 17.9 mg/100 g on day 12 and 12.6 mg/100 g on day 19, respectively.

Keywords: European eel; Nucleotides; Freshness indicators; *K*, K_i, *G*, *P*, *H* and F_r values; Biogenic amines

1. Introduction

The European eel is one of the most mysterious of fish and is found naturally around many coastal areas of Europe. It is believed that the breeding zone for the European eel is situated in the Sargasso sea. In Europe, total production of European eel (*Anguilla anguilla*) has increased from 7594 ton in 1996 to 10284 ton in 2001. The major production countries are the Netherlands, Denmark and Italy (FEAP, 2002). The white flesh, flavour and high flesh yield of eel are major attributes which attract consumers.

The quality of fish degrades after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage. As a result of these events, sensory quality and nutritional value of fish deteriorate. The concentrations of ATP and its breakdown products are used as indices of freshness in many fish species (Agustini et al., 2001; Chang, Chang, Shiau, & Pan, 1998; Greene, Babbitt, & Reond, 1990; Kyrana & Lougovois, 2002; Surette, Gill, & LeBlanc, 1988). The *K* value proposed by Saito, Arai, and Matsuyoshi (1959) is a biochemical index for fish quality assessment based on nucleotide degradation. The *K* value includes intermediate breakdown products and it varies within species of fish (Murata & Sakaguchi, 1986; Ryder, Buisson, Scott, & Fletcher,

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1984). Since adenosine nucleotides are almost entirely converted to IMP within 24 h post mortem (Jones, 1965), Karube, Matsuoka, Suzuki, Watanabe, and Toyama (1984) proposed the K_i value which excludes ATP, ADP and AMP. However, in some species, ATP, ADP and AMP remain even after 2 weeks (Karube et al., 1984). With some species the K_i value has been shown to increase very rapidly and then remain constant, even though freshness quality continues to decrease greatly (Greene & Bernatt-Byrne, 1990; Luong, Male, & Huynh, 1991). Therefore, the K value can be superior to the other values. The G value proposed by Burns, Ke, and Irvine (1985) was found to be superior to the K_i value for iced Atlantic cod, although it was observed to decrease during the first 2 or 3 days of iced storage, prior to its subsequent increase. In addition, H-values have been described by Luong, Male, Masson, and Nguyen (1992) as an index of freshness quality. The H value of iced Pacific cod was observed to increase steadily, indicating that it was superior to the K_i value (Luong et al., 1992). Gill, Thompson, Gould, and Sherwood (1987) also proposed the F_r value for yellow fin tuna. These results showed that measuring the concentration of the single nucleotide degradation product to determine freshness quality of seafood is not appropriate but measuring the concentration of ATP and its degradation products can be useful for determining freshness quality (Botta, 1995). The P value has been described by Shahidi, Chong, and Dunajski (1994). Determination of G and P values are useful with lean fish. However, it is difficult to obtain meaningful G and P values since fatty fish deteriorate due to rancidity (Shahidi et al., 1994). The rate of nucleotide degradation varies with body location (dark or white muscle), stress during capture, handling, season and storage conditions (Erikson, Beyer, & Sigholt, 1997; Luong & Male, 1992). Alasalvar, Taylor, and Shahidi (2002) indicated that the K, K_i, G, P and F_r values gave a better indication of cultured sea bream freshness than did the H value. In contrast, the H value gave a better indication than other values for wild sea bream.

The concentration of biogenic amines has been reported to be a reliable method of measuring freshness quality of fish, depending on species being examined (Mietz & Karmas, 1978; Rodríguez, Besteiro, & Pascual, 1999; Yamanaka, Shiomi, & Kikuchi, 1989). The formation of biogenic amines results from microbial degradation during the later stages of storage of fish. The shelf life and freshness quality of wild European eel, by sensory, chemical and microbiological (TVC) methods, were investigated in the first part of this study (Özogul, Özyurt, Özogul, Kuley, & Polat, in press). However, there is no information on the ratios of ATP and its breakdown products and biogenic amine formation in European eel. However, Morzel and van de Vis (2003) studied the effects of slauhtering methods on the quality of raw and smoked eels. They found that slaughter stress led to a higher K value. The goal of the second part of the study, therefore, was to determine the most appropriate freshness indicator(s) for assessing European eel quality and also to investigate the relationship between sensory assessments, freshness indicators and biogenic amine formation.

2. Materials and methods

2.1. Sample preparation and storage of eels

Eels (*A. anguilla*) purchased from a local fish processing company were 1-day post-capture on arrival at the laboratory in ice. Eels (average weight: 228.5 ± 21.98 g) were gutted, washed and divided into two lots in ice. One lot was stored in ice at a fish-to-ice ratio of 2:1 (w/w), and the second lot was stored in boxes without ice. All boxes were then stored in a refrigerator (3 ± 1 °C) for up to 19 days. Sensory and chemical analyses (nucleotide degradation products and biogenic amine) were performed on days 1, 5, 8, 12, 15, 19. Data were obtained using three different fish which were minced for each sampling.

2.2. Sensory analysis

For sensory analysis, triplicate samples, from each of the two storage conditions, were taken at regular intervals. Sensory analysis was assessed using the Tasmanian Food Research Unit Scheme (Branch & Vail, 1985) with modifications for eel. Sensory assesement procedure was described in the first part of the paper (Özogul et al., in press). Each assessment was carried out by a minimum of six trained panellists. Panellists were also asked to state whether or not the fish were acceptable. This was used to determine the shelf life of the eel.

2.3. Chemical analysis

2.3.1. ATP breakdown compounds

ATP and its degradation products were analysed using a rapid HPLC method (Özogul, Taylor, Quantick, & Özogul, 2000). The K, K_i, G, P, H and F_r values were calculated by the procedures described by Saito et al. (1959), Karube et al. (1984), Burns et al. (1985), Shahidi et al. (1994), Luong et al. (1992) and Gill et al. (1987), respectively. The formulas are as follows:

$$K\ (\%) = \left[\frac{(\mathrm{Ino} + \mathrm{HX})}{(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{Ino} + \mathrm{HX})}\right] \times 100,$$

$$K_i\ (\%)\left[\frac{(\mathrm{Ino}+\mathrm{HX})}{(\mathrm{IMP}+\mathrm{Ino}+\mathrm{HX})}\right]\times 100,$$

$$G\ (\%)\left[\frac{(\mathrm{Ino}+\mathrm{HX})}{(\mathrm{AMP}+\mathrm{IMP}+\mathrm{Ino})}\right]\times 100,$$

$$P\ (\%)\left[\frac{(\mathrm{Ino}+\mathrm{HX})}{(\mathrm{AMP}+\mathrm{IMP}+\mathrm{Ino}+\mathrm{HX})}\right]\times 100,$$

$$H\ (\%)\left[\frac{(\mathrm{HX})}{(\mathrm{IMP}+\mathrm{Ino}+\mathrm{HX})}\right]\times 100,$$

$$F_r\ (\%)\left[\frac{(\mathrm{IMP})}{(\mathrm{IMP}+\mathrm{Ino}+\mathrm{HX})}\right]\times 100.$$

2.3.2. Biogenic amines

Biogenic amines were analysed using an HPLC method (Özogul, Taylor, Quantick, & Özogul, 2002a). Benzoyl chloride, as a derivatization reagent, was used and the derivatization procedure was based on that of Redmond and Tseng (1979).

2.4. Apparatus

High-performance liquid chromatography (HPLC) used Shimadzu LC-10VP (Shimadzu, Kyoto, Japan) apparatus equipped with a UV/VIS detector (Spectra-Physics SP 8450, Analytical Inc., UK) and a low gradient pump (Shimadzu LC-10ATVP) with a four channel mixer (Shimadzu FVC-10ALVP). For biogenic amine analysis, the column was reverse-phase, C18, nucleosil, 250 × 4.6 mm, particle diameter 5 μm (Mecherey–Nagel, Duren, Germany) For nucleotide determination, the column was a Sphereclone ODS 2 C18, 150 × 4.60 mm, particle diameter 5 μm. (Phenomenex, Macclesfield, Cheshire, UK).

2.5. Statistical analysis

For data analysis, standard deviation and ANOVA were used. Significance of differences was defined at $P < 0.05$. Statistical comparison was based on three samples for each specific storage time.

3. Results and discussion

3.1. Sensory assessment

As indicated in our first part of this study (Özogul et al., in press), the limit for acceptability of eel stored in ice was ~12–14 days, and ~5–7 days in boxes without ice at 3 °C. Although the initial sensory scores for the two storage conditions were the same on day 1, these scores for fish stored in boxes without ice were significantly higher ($P < 0.05$) than for fish stored in ice at day 8 and 12.

3.2. ATP and its breakdown products

The patterns of ATP breakdown products in European eel stored in ice and at 3 ± 1 °C are shown in Figs. 1 and 2, respectively. The main changes are the loss of IMP and the increase in Hx with storage. ATP, ADP and AMP levels were very low (less than 0.25 μmol/g) at the beginning of the storage period and then decreased over the storage period for eel stored in both ice and in boxes without ice. The degradation of IMP and formation of Hx appeared to be more rapid at 3 ± 1 °C than in ice.

IMP is a flavour enhancer and is strongly associated with acceptability in fish (Bremner, Olley, Stratham, & Vail, 1988; Fletcher & Statman, 1988). There were significant differences ($P < 0.05$) in IMP levels between eel stored in ice and eel kept in boxes without ice, except

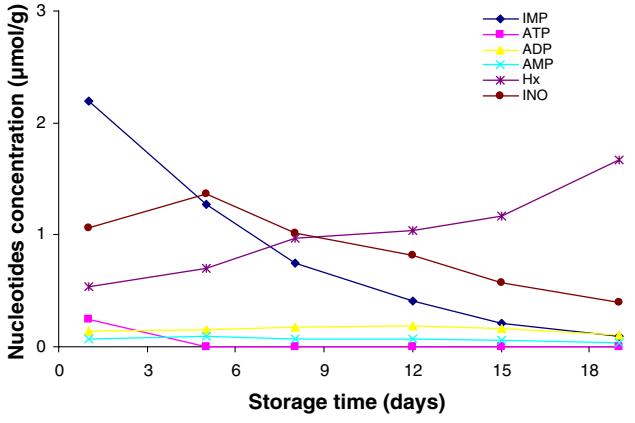

Fig. 1. Changes in concentration of ATP breakdown compounds in European eel stored in ice. Each point represents the mean value of three determinations.

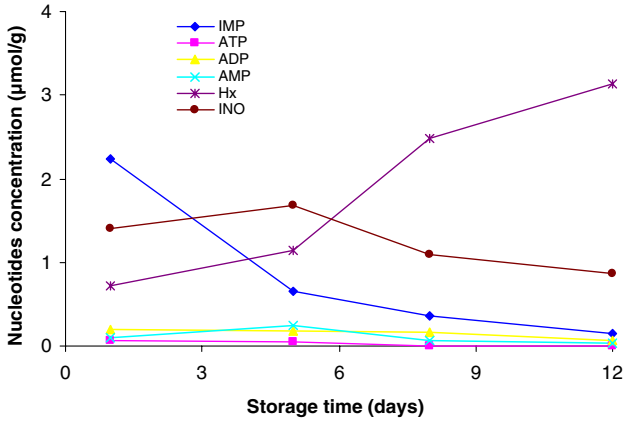

Fig. 2. Changes in concentration of ATP breakdown compounds in European eel stored in boxes without ice (3 ± 1 °C). Each point represents the mean value of three determinations.

on day 1. The degradation rate of IMP was slower in eel stored in ice than in those stored in boxes without ice. The initial level of IMP in eel stored in ice and in boxes without ice was around 2.2 μmol/g and decreased sharply to 0.75 μmol/g on day 8 and 0.66 μmol/g on day 5, respectively. After that, the level of IMP decreased slowly until day 19 (0.09 μmol/g) for eel stored in ice and day 12 (0.14 μmol/g) for eel kept in boxes without ice. However, in European sea bass, the concentration of IMP increased at first (7.05 μmol/g) and then declined steadily throughout the storage period (Kyrana & Lougovois, 2002). Ehira and Uchiyama (1987) reported that the IMP level in Pacific cod and Alaska pollack declined to less than 1 μmol/g in 2 days.

During the metabolism of ATP post mortem, hypoxanthine (Hx) is formed, which is bitter and is regarded as a contributor to off-flavours. Hypoxanthine (Hx) has been reported as an index of freshness of fish, but the accumulation of Hx varies, both within given species and between species (Huss, 1988), and even with body location (Murata & Sakaguchi, 1986). In this study, Hx concentration increased with the increase of storage period, as reported for other species (Alasalvar et al., 2002; Greene et al., 1990; Kyrana, Lougovois, & Valsamis, 1997; Özogul et al., 2000; Price, Melvin, & Bell, 1991). There were significant differences ($P < 0.05$) in the level of Hx between two storage conditions, except on day 1. The initial levels of Hx in eel stored in ice and in boxes without ice were 0.54 and 0.75 μmol/g on day 1 and increased to maximum levels of 1.67 and 3.15 μmol/g at the end of the storage period, respectively.

The concentrations of INO in eel stored in ice and in boxes without ice were 1.07 and 1.40 μmol/g, respectively. On day 5, its levels increased to 1.68 for eel kept in boxes without ice and 1.36 μmol/g for eel stored in ice and then decreased steadily for both storage conditions.

3.3. K and related values

The freshness indicators (K, K_i, G, P, H and F_r) of European eel stored in ice and in boxes without ice were determined from the concentrations of nucleotides and are shown in Figs. 3 and 4, respectively. K and related values increased linearly (except F_r value) with storage time in European eel stored under both conditions. Linear regressions (r^2) obtained from K, K_i, G, P, H and F_r were 0.95, 0.96, 0.83, 0.96, 0.99 and 0.96, respectively, for eels stored in ice whereas, for eels kept in boxes without ice, the values were 0.86, 0.86, 0.96, 0.91, 0.98 and 0.86, respectively. The lowest value obtained was for G value of eels in ice. For eels kept in boxes without ice, linear regressions of K, K_i and F_i values were found to be low. The reason for this could be a rapid decrease in IMP and a sharp increase in Hx in eel, thus loading to the rapid increase of K, K_i and F_i values. In addition, nucleotide degradation proceeds faster at high temperature than during storage in ice (Özogul, Polat, & Özogul, 2004; Rodríguez et al., 1999).

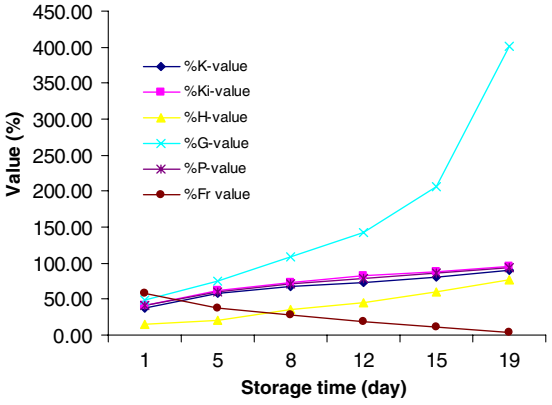

Fig. 3. K, K_i, G, P, H and F_r value changes of European eel stored in ice. The r^2 values of linear regressions are 0.95 (K), 0.96 (K_i ,P, F_r), 0.83 (G), and 0.99 (H) with time.

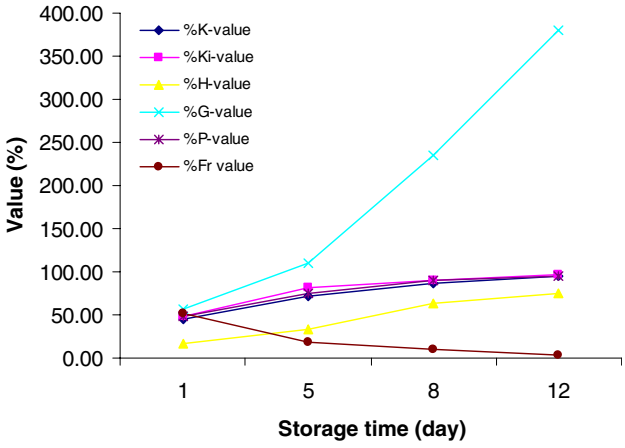

Fig. 4. K, K_i, G, P, H and F_r value changes of European eel stored in boxes without ice (3 ± 1 °C). The r^2 values of linear regressions are 0.86 (K, K_i and F_r), 0.96 (G), 0.91 (P), and 0.98 (H) with time.

When eels stored in ice and in boxes without ice were considered, at the limit of acceptability, by assessors on ~12–14 days and ~5–7 days, respectively, the average K, K_i and P values were ~70–85%. H values were ~60% and F_r values were ~10% for both storage conditions. These values were higher than those reported for cultured and wild sea bream stored in ice (Alasalvar et al., 2002; Alasalvar, Taylor, Öksüz et al., 2001) and sardine stored in air (Özogul et al., 2004). The highest value in this study was obtained for the G value (~200%) at the time of the acceptability limit for eels stored in ice and in boxes without ice. However, the best indicators for freshness of eel in ice were the K, K_i, P, H and F_r values. Significant differences ($P < 0.05$) were found in the K and related values between eels in ice and eels kept in boxes without ice over the storage period, except for H and G values ($P > 0.05$). The mean initial the K

values of eels were high, indicating that eels were subjected to handling stress before or during slaughtering. A rapid rise in the *K* value in stressed fish has been reported (Erikson et al., 1997; Lowe, Ryder, Carragher, & Wells, 1993; Morzel & van de Vis, 2003; Sigholt et al., 1997). *K* values increased with storage time, reaching 90% and 94% from the initial value of 38% and 45% in eels stored in ice and in boxes without ice during 19 days of storage, respectively. The *K* value provided a useful indicator of freshness in eels stored in ice. Similar results were found with sockeye salmon, Pacific herring, Pacific cod, sea bream, sardine and herring stored in ice (Alasalvar et al., 2002; Huynh, Mackey, & Gawley, 1992; Luong et al., 1991; Özogul et al., 2004).

3.4. Biogenic amines

The biogenic amine content of fish is useful for estimating the freshness and degree of spoilage of fish and fish products. These amines are found in fresh fish at low levels and their presence is associated with bacterial spoilage (Duflos, Dervin, Malle, & Bouquelet, 1999; Fernández-Salguero & Mackie, 1979). The biogenic amine content of fish depends on fish species, free amino acid content (Mackie, Pirie, Ritchie, & Yamanaka, 1997), the moment of capture and stomach contents at death, since microbial flora vary seasonally (Rodríguez et al., 1999).

The concentrations of the biogenic amines in the muscle of eel kept in ice, and in boxes without ice are given in Tables 1 and 2. Among the biogenic amines, histamine is potentially hazardous and is believed to be the causative agent in Scombroid poisoning (Arnold & Brown, 1978). In the present study, histamine produced by bacterial decarboxylation of free histidine (Fernández-Salguero & Mackie, 1979) was detected in the samples. In this experiment, the level of histamine became higher than the hazardous concentration (5 mg/100 g fish-legal limit for histamine set by the US Food and Drug Administration; FDA, 1996) in eel stored without ice after 6–7 days and, in ice, after 13–14 days of storage. However, the level of histamine did not develop beyond the limit of 20 mg/100 g set by the EU (EEC, 1991) for both storage conditions. The levels of histamine in the muscle of eel kept in boxes without ice and in ice increased to maximum levels of 17.9 mg/100 g on day 12 and 12.6 mg/100 g on day 19, respectively. There were significant differences ($P < 0.05$) between the histamine levels of eels stored in ice and eels in boxes without ice on days 8 and 12. It has also been reported that the concentration of histamine in fish increased with an increase in the temperature of storage (Dawood, Karkalas, Roy, & Williams, 1988; Özogul, Taylor, Quantick, & Özogul, 2002b; Ritchie & Mackie, 1980; Rodriquez-Jerez, Lopez-Sabater, Hernandez-Herrero, & Mora-Ventura, 1994), as found in this study.

Putrescine, cadaverine and spermidine levels increased throughout the storage period,with significantly higher increase ($P < 0.05$) in eel stored in boxes without ice (Tables 1 and 2). Valle, Malle, and Bouquelet (1996) found that, when the fish were inedible, putrescine and cadaverine contents of herring stored at 0 °C were 1.01 and 2.3 mg/100 g, respectively, whereas plaice and whiting contained 1.57 and 5.8 mg/100 g putrescine and 9.1 and 9.2 mg/100 g cadaverine, respectively. In this study, when eels in ice (on day 15) and in boxes without ice (on day 8) were rejected by the sensory panel, the levels of putresine were 4.64 and 8.77 mg/100 g and cadaverine levels were 3.04 and 8.68 mg/100 g, respectively.

However, spermine, agmatine and tyramine levels fluctuated during the storage period and agmatine in the muscle of eel stored in ice was not detected until 8 days and, after that, the level rose to 2.04 mg/100 g on day 19. The concentrations of tryptamine and 2-phenylethylamine were negligible in eels stored under the two conditions.

Fish contain trimethylamine oxide (TMAO) and the quantity depends on fish species and the environment. TMA is associated with the fishy odour of spoilage and is part of the spoilage pattern of many fish. Seawater fish contain 1–100 mg TMAO in every 100 g of muscular tissue whereas freshwater fish generally contain only 5–20 mg/100 g (Stansby & Olcott, 1963, ch. 26). Tezkeredzic and Pfeifer (1987) found an upper acceptable limit of TMA in trout for human consumption of

Table 1
The concentrations of biogenic amines (mg/100 g) during ice storage of eel

Storage days	HIS	PUT	CAD	SPD	SPN	AGM	TYR	TRYP	2-PHENY	TMA
1	0.40 ± 0.69	0.86 ± 0.17	0.32 ± 0.11	0.85 ± 0.28	2.09 ± 0.51	–	0.38 ± 0.25	0.26 ± 0.25	0.32 ± 0.30	0.79 ± 0.37
5	1.55 ± 0.74	1.28 ± 0.26	1.05 ± 0.23	1.01 ± 0.13	2.45 ± 1.47	–	0.94 ± 0.54	0.34 ± 0.16	0.50 ± 0.44	1.44 ± 1.31
8	2.07 ± 1.82	2.86 ± 1.37	1.70 ± 0.11	1.38 ± 0.35	3.26 ± 0.03	–	0.90 ± 0.79	–	0.44 ± 0.27	2.34 ± 1.05
12	3.84 ± 0.35	3.77 ± 0.63	1.84 ± 0.37	1.56 ± 0.37	1.48 ± 0.94	1.28 ± 1.21	0.96 ± 0.36	0.22 ± 0.19	0.20 ± 0.14	7.30 ± 3.49
15	7.13 ± 1.58	4.64 ± 1	3.04 ± 0.13	1.89 ± 0.22	1.95 ± 1.07	1.35 ± 1.33	1.04 ± 2.52	0.48 ± 0.28	–	8.51 ± 2.44
19	12.6 ± 0.09	5.48 ± 1.10	3.99 ± 0.19	2.20 ± 0.59	1.75 ± 0.34	2.04 ± 1.5	0.65 ± 0.30	0.16 ± 0.07	0.38 ± 0.34	9.75 ± 1.22

The values are expressed as means (±standard deviation), *n*: 3.
HIS, histamine; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPN, spermine; AGM, agmatine; TYR, tyramine; TRYP, tryptamine; 2-PHENY, 2-phenylethylamine; TMA, trimethylamine; –, not detected; ±, standard deviation.

Table 2
The concentrations of biogenic amines (mg/100 g) in eel kept in boxes without ice (at 3 ± 1 °C)

Storage days	HIS	PUT	CAD	SPD	SPN	AGM	TYR	TRPT	2-PHENY	TMA
1	0.85 ± 0.47	1.18 ± 0.15	0.69 ± 0.24	1.70 ± 0.11	2.86 ± 0.84	0.66 ± 1.14	1.26 ± 0.65	0.19 ± 0.15	0.65 ± 0.59	2.53 ± 2.84
5	1.04 ± 0.25	3.05 ± 0.30	5.20 ± 0.61	2.04 ± 0.25	1.98 ± 0.18	0.94 ± 1.63	0.41 ± 0.14	0.38 ± 0.34	0.40 ± 0.32	5. 66 ± 2.92
8	6.60 ± 2.06	8.77 ± 1.22	8.68 ± 0.91	3.33 ± 0.40	3.80 ± 0.15	2.03 ± 1.76	1.57 ± 2.59	0.72 ± 0.62	0.70 ± 0.43	7.71 ± 2.09
12	17.9 ± 5.40	13.7 ± 3.11	11.1 ± 1.29	10.8 ± 0.91	4.57 ± 0.56	1.28 ± 1.21	2.10 ± 0.33	–	0.90 ± 3.05	11.5 ± 3.2

The values are expressed as means (±standard deviation), *n*: 3.

around 10 mg/100 g. Rodríguez et al. (1999) reported that farmed rainbow trout contained 52.5 mg/100 g of TMAO, due to the diet, which was based on pellets containing seafish. They found that TMA concentration increased during storage, reaching 0.6 mg/100 g in gutted trout.

TMA is not produced in significant amounts at the early stages of chill storage of fish but it appears after three or four days, after which the rate of production of TMA parallels the bacterial proliferation pattern (Lindsay, 1994). Therefore, it is not considered suitable for fish stored in ice for less than 6 days (Howgate, 1982). Fresh fish has very low amounts of TMA, with values below 1.5 mg TMA/100 g in fresh cod, but values increase during spoilage. The fish is considered stale when TMA is above 30 mg/100 g cod (Bonnell, 1994). The level of TMA found in fresh fish rejected by sensory panels varies between species, but is around 10–15 mg TMA-N/100 g in aerobically stored fish (Dalgaard, Gram, & Huss, 1993). In this study, when eels in ice were rejected by the sensory panel (~12–14 days), the level of TMA was between 7.30 and 8.51 mg/100 g whereas, in eels kept in boxes without ice (~5–7 days), its level was between 5.66 and 7.71 mg/100 g. The concentration of TMA increased during storage, reaching 9.75 mg/100 g in eels stored in ice and 11.5 mg/g in eels kept in boxes without ice.

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