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Biogenic amine production and nucleotide ratios in gutted wild sea bass (*Dicentrarchus labrax*) stored in ice, wrapped in aluminium foil and wrapped in cling film at 4 °C

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Abstract

Biogenic amine profiles and nucleotide ratios for wild sea bass (*Dicentrarchus labrax*) stored in ice, in boxes without ice, wrapped in aluminium foil (WAF) and wrapped in cling film (WCF) at 4 °C were studied. Ten biogenic amines (histamine putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, 2-phenylethylamine, agmatine and serotonin) and nucleotide ratios (K-value, Ki-value, H-value and G-value) were determined. The mean value of K, Ki, H and G was 66%, 72%, 13% and 81%, respectively, when the fish reached the limit of acceptability to the panellists. Linearity (r^2) of K, Ki, H and G values for all storage conditions was 0.94–0.96, 0.94–0.96, 0.88–094 and 0.94–0.98, respectively. The mean values of K, Ki and G were higher in WCF than WAF and fish stored in ice except on day 8. The highest K, Ki and G values were obtained from fish wrapped in cling film, followed by fish in aluminium foil and fish stored in ice. Histamine was detected only towards the end of the storage time for WAF and WCF. As storage time progressed, cadaverine, spermidine and spermine became the dominant amines reaching 11, 8.9 and 10 mg/kg, respectively, at days 12 of storage in ice. Putrescine and tryptamine contents also rose steadily to reach 5.8 and 2.1 mg/kg, respectively. The levels of biogenic amines were significantly higher in sea bass stored in WCF and WAF as compared with the iced storage conditions.

Keywords: Sea bass; Biogenic amines; Nucleotide degradation; Cling film; Aluminium foil

1. Introduction

Sea bass (*Dicentrarchus labrax*) production is expanding rapidly in Europe to meet internal and external demand. The production of sea bass was 19,402 metric tons in 1996 and reached 60,451 tons in 2002. Greece was the largest producer (28,000 tons), followed by Turkey (15,500 tons), Italy (9000 tons), France (3500 tons) and Spain (3180 tons). Portugal, Cyprus, and Iceland had also a small production (FEAP, 2002). Sea bass is an economically important fish species in many Mediterranean countries because of its high nutritional quality and excellent sensory properties (desirable aroma and taste). It is produced not only for domestic consumption but also for export markets. Sea bass are generally sold as whole, gutted or filleted products for immediate consumption at the retail market.

Biogenic amines are usually generated by microbial decarboxylation of specific free amino acids in fish or shellfish tissue (Rawles, Flick, & Martin, 1996). The importance of determining the concentrations of biogenic amines in fish and fish products is related to their impact on human health and food quality. The

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most significant biogenic amines occurring in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine. Histamine is formed in fish during bacterial spoilage due to decarboxylation of the amino acid histidine by bacteria with the enzyme histidine decarboxylase. Arginine is easily converted to agmatine as result of bacterial activity. Lysine can be converted by bacterial action into cadaverine. Tyramine, tryptamine and 2-phenylethylamine can be formed from tyrosine, trytophan and phenylalanine, respectively. Putrescine is the precursor of ornithine. Among the biogenic amines, histamine is potentially hazardous and the causative agent of histaminic intoxication associated with the consumption of seafood (Morrow, Margolis, Rowland, & Roberts, 1991). Cadaverine and putrescine have been reported to enhance the toxicity of histamine (Taylor, 1985). Histamine is the only biogenic amine for which maximum legal levels (10 mg/100 g) have been established by the European Union for tuna and other fishes of the Scombridae and Scomberesocidae families (EEC, 1991). Due to the high possibility of histamine toxicity in pelagic fish, the Food and Drug Administration (FDA) has lowered the allowed histamine level to 5 mg/100 g (FDA, 1996).

Fish muscle is able to support the bacterial formation of a wide variety of amines that come from the decarboxylation of amino acids. Biogenic amines are produced at very low levels in fresh fish, and their formation is related to bacterial spoilage (Fernandez-Salguero & Mackie, 1987). As the amines are produced by spoilage bacteria towards the end of shelf life, their levels are considered as indices of spoilage rather than freshness (Mackie, Pirie, Ritchie, & Yamanaka, 1997). Dawood, Karkalas, Roy, and Williams (1988) reported that putrescine and cadaverine could be used to assess the freshness of rainbow trout stored at chill temperature. Based on the content of the histamine, cadaverine, putrescine, spermine and spermidine in canned tuna, a chemical quality index was proposed by Mietz and Karmas (1977). In addition, biogenic amines were found to be useful as quality index of fish decomposition (Yamanaka, Shimakura, Shiomi, & Kikuchi, 1986). Yamanaka, Shiomi, and Kikuchi (1987) also indicated that agmatine might be used as an index for freshness of common squid.

Significant variations both among and within species clearly limit the practical usefulness of measuring the level of a single nucleotide breakdown product or a single nucleotide ratio to measure freshness quality (Greene, Babbitt, & Reppond, 1990). This important variation in the rate of nucleotide degradation between fish species is probably caused by variation in the intrinsic enzyme activity and nucleotide concentrations (Hiltz, Dyer, Nowlan, & Dingle, 1971). Measuring the concentration of nucleotide degradation compounds as combinations can be a useful way of determining freshness. Therefore, the K value proposed by Saito, Arai, and Matsuyoshi (1959), includes intermediate breakdown products. Due to the rapid disappearance of adenosine phosphate, Karube, Atsuoka, Sufzuki, Watanabe, and Toyama (1984) proposed the Ki value which excludes ATP, ADP and AMP. In addition, H-values (Luong, Male, Masson, & Nguyen, 1992) and G-value (Burns, Ke, & Irvine, 1985) have been described as an index of freshness quality due to the wide diversity in pattern of nucleotide catabolism.

Packaging materials such as aluminium foil and cling film provide good protection, easier handling, and better presentation. Aluminium foil and cling film as packaging material have certain properties that are very convenient to use for food. Aluminium foil is resistant to odour, water, air, light and oil. It possesses high thermal and electrical conductivity properties as well. Not only its strength and durability is very high and but also its lamination and coating is very easy. Cling film is a hygienic packaging material for food wrapping and protection. It is very convenient for use in freezers, refrigerators and microwave ovens. It keeps flavours, freshness and taste inside and avoids mixing odours. It also provides useful protection from dust, moisture, fungi.

There is a considerable amount of information available on the formation of amines in fish such as haddock, herring, rainbow trout, mackerel, tuna, scallop (Dawood et al., 1988; Fernandez-Salguero & Mackie, 1987; Klausen & Lund, 1986; Mackie et al., 1997; Özogul, Taylor, Quantick, & Özogul, 2002b). However, limited information exists regarding the production of biogenic amines in wild sea bass stored in ice, aluminum foil and cling film. Therefore, the aims of this study were to investigate the formation of biogenic amines and nucleotide ratios (K, Ki, H and G values) in sea bass stored in ice, in boxes without ice, wrapped in aluminium foil (WAF) and wrapped in cling film (WCF) at 4 °C. Another aim of the present study was to investigate the potential usefulness of nucleotide ratios and biogenic amines as a freshness quality index or spoilage index during storage.

2. Materials and methods

2.1. Packaging and storage of sea bass

Wild sea bass (*D. labrax*) used in the present study were caught by academic staff of Fisheries Faculty in the lagoon of the Mediterranean Sea and iced immediately after harvest. On arrival at the laboratory, the fish were gutted and divided into four lots. One lot was stored in ice, the second lot was stored in boxes without ice and the remaining two lots were wrapped

individually in aluminium foil and cling film. All samples were stored in a refrigerator with the controlled temperature at 4 °C. At regular intervals, three fish were removed from each storage condition for biogenic amine and nucleotide degradation product analyses.

2.2. Sensory analyses

The sensory assessment of sea bass was carried out using the modified Tasmanian Food Research sensory assessment scheme (Branch & Vail, 1985). The sensory assessment test was given in more details in the first part of this study (Özogul, Gökbulut, Özyurt, Özogul, & Dural, 2005). Triplicate samples from each of the four storage conditions were taken at regular intervals for sensory analysis.

2.3. Analytical method

ATP and its breakdown products were analysed according to method of Özogul, Taylor, Quantick, and Özogul (2000). Biogenic amines analysis was determined using the method of Özogul, Taylor, Quantick, and Özogul (2002a).

2.4. Apparatus and columns

High-performance liquid chromatography (HPLC) analyses 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, FCV-10ALVP). For the biogenic amine analyses, the column was reverse-phase, C18, nucleosil, 250×4.6 mm, particle diameter 5 μm (Mecherey-Nagel, Duren, Germany). For nucleotides determination, the column was a Sphereclone ODS 2 C_{18}, 150×4.60 mm, particle diameter 5 μm. (Phenomenex, Macclesfield, Cheshire, UK).

2.5. Reagents

All nucleotides and biogenic amines standards were purchased from Sigma-Aldrich. For histamine and nucleotide analysis, the mobile phase consisted of acetonitrile and HPLC grade water.

2.6. Nucleotides analysis

K, Ki, H and G values were calculated by the procedures described by Saito et al. (1959), Karube et al. (1984), Luong et al. (1992) and Burns et al. (1985), respectively. In this study, the K-value, Ki- value, H-value and G-value were expressed as a percentage and the formulae used are as follows;

$$\text{K-value}(\%) = (\text{Hx}+\text{INO})/(\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{Hx}+\text{INO}) \times 100$$

$$\text{Ki-value}(\%) = (\text{Hx}+\text{INO})/(\text{IMP}+\text{Hx}+\text{INO}) \times 100$$

$$\text{H-value}(\%) = (\text{Hx})/(\text{IMP}+\text{Hx}+\text{INO}) \times 100$$

$$\text{G-value}\ (\%) = (\text{Hx}+\text{INO})/(\text{AMP}+\text{IMP}+\text{INO}) \times 100$$

where ATP, adenosine triphosphate; ADP, adenosine diphospahte; AMP, adenosine monophosphate; IMP, inosine monophosphate; INO, inosine; Hx, hypoxanthine.

2.7. Sample preparation for biogenic amine analyses

A rapid HPLC method was used for biogenic amine determination. Fish muscle (5 g) was taken from the dorsal part of the fish fillet without skin and transferred to a 250 ml centrifuge tube. The sample was then homogenised with 20 ml 6% TCA for 3 min, centrifuged at 11,180*g* for 10 min at 4 °C and filtered through Whatman filter paper (No: 1). The aliquot was made up to 50 ml with distilled water and was stored in a freezer (−18 °C).

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For derivatization of standard amine solutions, 50 μl was taken (2 ml for extracted fish samples) from each free base standard solution (10 mg/ml). One milliliter of 2 M sodium hydroxide was added, followed by 1 ml benzoyl chloride (2%), vortex mixed for 1 min. The reaction mixture was left at room temperature for 5 min and then centrifuged for 10 min. After that, the benzoylation was stopped by adding 2 ml of saturated sodium chloride solution and the solution extracted 2 times with 2 ml of diethyl ether. The upper organic layer was transferred into a clean tube after mixing. Afterwards, the organic layer was evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500 μl of acetonitrile and 5 μl aliquots were injected into the HPLC.

2.8. Statistical analysis

For statistical analysis, student *t*-test and standard deviation were used. Significance of differences was defined at $P < 0.05$. Statistical comparison was based on 3 samples for each treatment for each specific storage time.

3. Results and discussion

3.1. Nucleotide ratios (K, Ki, H and G values)

Figs. 1a–1d show the patterns of K, Ki, H and G values for sea bass stored in boxes at chill temperature (4 °C), stored in ice, wrapped in cling film and wrapped in aluminium foil. The initial K, Ki, H and G values

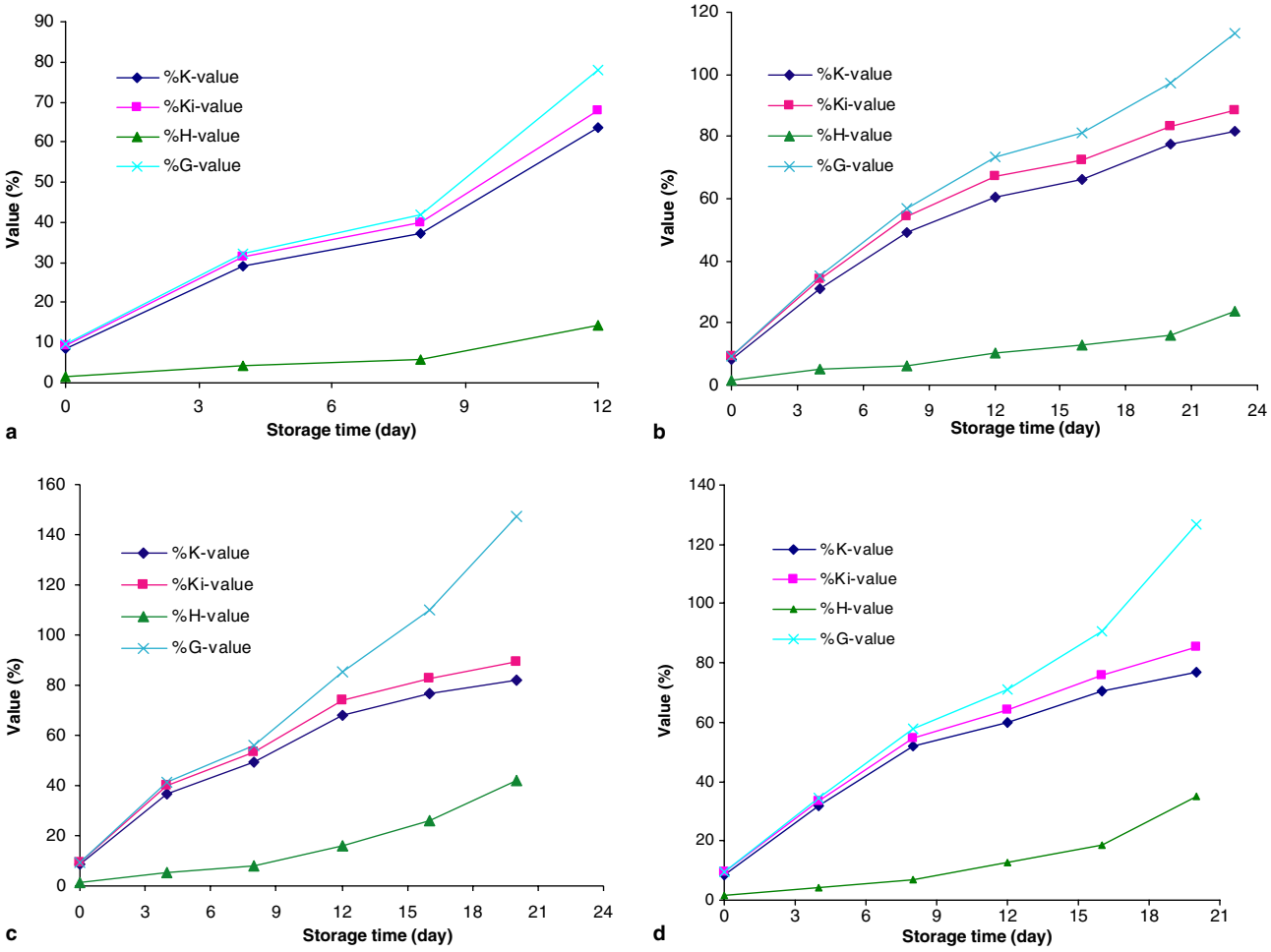

Fig. 1. (a) Mean values of sea bass stored at 4 °C. (b) Mean values of sea bass stored in ice. (c) Mean values of sea bass wrapped in cling film at 4 °C. (d) Mean values of sea bass wrapped in aluminium foil at 4 °C.

were found to be 8.5%, 9.3%, 1.3% and 9.5%, respectively, and they increased continuously over the storage period. The final values of K, Ki, H and G were found to be 64–82%, 68–89%, 14–41% and 78–147% for all storage conditions, respectively. The rates of increase of K and related values are similar except for that of the H-value which rises very slowly during the storage period. Linearity (r^2) of K, Ki, H and G values for all storage conditions was 0.94–0.96, 0.94–0.96, 0.88–094 and 0.94–0.98, respectively. Alasalvar, Taylor, Oksuz, Shahidi, and Alexis (2002) reported that linear regression for K, Ki and G values were 0.98 or higher but for the H values were 0.93 and 0.83 for cultured and wild sea bass, respectively. There is no considerable trend in the changes of H value during the first 8 days of storage because of steady rise in the concentration of Hx. This is in agreement with the study of Alasalvar et al. (2002).

The rapid increase of the K and related values is entirely due to the sharp drop of IMP in the fish flesh. The loss of IMP through degradation to INO and Hx would cause a loss of fresh fish desirable compounds. As organoleptic quality of sea bass declined, the K and related values increased. Sensory analysis showed that the limit of acceptability of wild sea bass was 16 days for iced storage, 4 days for without ice storage, 8 days for aluminium foil and cling film storage. Mean values of K, Ki, H and G at the limit of acceptability were about 29%, 31%, 4% and 32%, respectively.

Changes of mean K, Ki, H and G-values for sea bass stored in ice are given in Fig. 1b. The K, Ki, H and G values were 77%, 83%, 16% and 97%, respectively, at the point of sample rejection on day 20 of ice storage. When the fish reached the limit of acceptability to panellists for fish stored in ice, the mean values of K, Ki, H and G were 66%, 72%, 13% and 81%, respectively, on day 16. Alasalvar et al. (2002) reported that the average K, Ki, and G values in wild sea bass stored in ice was 60–70% and H value was 10–15% when the wild sea bass reached the limit of acceptability on day 16 to 18. Significant differences ($P < 0.05$) were observed between (K, Ki, G) and H values. No significant differences

($P > 0.05$) were found between K and Ki values although ATP, ADP and AMP content were excluded from the calculation of Ki-value.

Fig. 1c illustrates changes of mean K, Ki, H and G values in sea bass wrapped in cling film. The level of K, Ki, H and G value increased continuously and reached 82%, 89%, 41.9% and 147%, respectively, at the end of storage time. The organoleptically perceived quality was lost after 8 days which correlated well with the 50% K value, 53% Ki value, 8% H value and 56% G value. Rodriguez, Besteiro, and Pascual (1999) found that the Ki-value of vacuum-packed trout was 90% on the day of marginal acceptability.

Yasuda, Nishino, Chiba, Nakano, and Yokoyama (1989) found that yellowtail fillets in MAP (60% N_2 and 40% CO_2) had a lower K value than the stretch-wrap-packaged yellowtail stored at the same temperature (5 °C). Hattula and Kiesvaara (1996) reported that K-value is a reliable indicator of freshness that is applicable for frozen fish, smoked fish and fish stored under modified atmosphere. However, no effect of the modified atmosphere packing on the K values was reported by Lopez-Galvez, De la Hoz, Blanco, and Ordonez (1998) in sole fillets. Reddy et al. (1997) also found that, K values of MAP stored fillets of catfish at 4, 8 and 16 °C storage increased gradually during early and middle storage time and decreased towards end of storage period with sensory spoilage, indicating no relationship between sensory spoilage and K value.

The pattern of K, Ki, H and G values for sea bass stored in aluminium foil is illustrated in Fig. 1d. H-values increased slowly during the early storage but a sharp increase was observed towards the end of storage period. At the point of sensory rejection, the H-value was found to be 12.4%. The mean values of K, Ki and G were 4 higher in WCF than WAF and fish stored in ice except on day 8. The highest K, Ki and G values were obtained from fish wrapped in cling film, followed by fished wrapped aluminium foil and fish stored in ice. This shows that wrapping the fish in cling film and aluminium foil is not advantageous compared to fish stored in ice. This shows that lower temperature was more effective than wrapping fish in cling film and aluminium foil.

CO_2 has an inhibitory effect on micro-organisms. Oxygen pressures in a cling film will be rapidly reduced due to the microbial activities since some aerobic micro-organisms consume oxygen according to their number and nature. In the later stages of storage, the level of CO_2 will rise in this packing system. In the first part of the study (Özogul et al., 2005), microbiologic results showed that bacterial load (total viable counts) was lower in WCF than WAF storage condition. Therefore, inhibition of the bacterial growth results in reducing the concentration of INO and Hx. This would directly decrease the mean value of K, Ki, H and G. There was surprisingly no relationship found between INO and Hx concentration and bacterial growth. Although bacterial growth was inhibited by cling film, K, Ki, H and G value were higher under WCF than WAF storage conditions. Considerable differences were observed between sea bass stored in ice and WCF storage condition except day 8.

A loss of freshness in fish can be objectively measured by changes of K and related values. K values were found to be similar to Ki values throughout the storage period. However, H values of sea bass have been observed to increase slowly during the initial storage period compared to K and Ki values, although the freshness continued to decrease greatly. The G value rose gradually until 12 days of storage after which it increased sharply due to the increase in Hx content. It can be inferred that values of K, Ki are superior to H value and G-value and provide useful freshness indicators for all storage conditions. No significant differences ($P > 0.05$) were found among K, Ki and G value, but there were significant differences ($P < 0.05$) between H value and K, Ki and G value throughout the storage period.

3.2. Biogenic amine formation

The concentrations of the biogenic amines present in the muscle of sea bass stored in boxes at chilled temperature are given in Tables 1–4. The storage periods were prolonged well beyond the accepted period for edibility to give a full picture of the formation of these amines from the fresh to decayed condition. Although ten biogenic amines were studied namely, histamine, putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, 2-phenylethylamine, agmatine and serotonin, three amines (tyramine, serotonin and agmatine) were not detected in any of the fish samples during the storage period. The amines present in muscle of sea bass showed low initial concentrations for all storage conditions. Histamine was not found during the storage of sea bass in boxes at chilled temperature (4 °C). As storage time progressed, cadaverine, spermidine and spermine became the dominant amines reaching 11, 8.9 and 10 mg/kg, respectively, at 12 days of storage in ice. Putrescine and tryptamine contents also rose steadily to reach 5.8 and 2.1 mg/kg, respectively, after 12 days storage. The other amines studied were all at concentrations less than 1 mg/kg. Poli et al. (2001) found that concentration of the putrescine, cadaverine, histamine, spermine and spermidine in reared sea bass stored at 4 °C was 4.8, 2.9, 4.3, 5.4 and 12 mg/kg, respectively, when the fish was unfit for consumption.

The changes in biogenic amine contents in the muscle of sea bass stored in ice are shown in Tables 2. The lowest level of the biogenic amines was obtained from sea bass stored in ice. Storage of the sea bass in ice lowered the concentration of biogenic amines compared to the other storage conditions. Histamine was not detected

Table 1
Biogenic amines content (mg/kg muscle) in sea bass stored at 4 °C

Storage time (days)	PUT	CAD	SPMD	SPM	TRPT	2-FNLE	HST
0	0.40 (0.10)		1.59 (1.18)	1.60 (1.02)	0.50 (0.29)	0.20 (0.18)	
4	0.61 (0.29)	0.15 (0.10)	1.69 (0.54)	5.00 (2.10)	0.20 (0.15)		
8	1.10 (0.33)	0.46 (0.30)	2.94 (0.67)	7.90 (1.54)	0.40 (0.35)	0.40 (0.32)	
12	5.85 (2.65)	11.0 (2.83)	8.96 (3.93)	10.0 (2.79)	2.10 (0.95)	0.60 (0.36)	

The values are expressed as mean (standard deviation), ($n = 6$).
PUT: Putrescine, CAD: Cadaverine, SPMD: Spermidine, SPM: Spermine, TRPT: Tryptamine, 2-FNLE: 2-phenylethylamine, HIS: Histamine. Agmatine, Serotonin and Tyramine were not detected.

Table 2
Biogenic amines content (mg/kg muscle) in sea bass stored in ice

Storage time (days)	PUT	CAD	SPMD	SPM	TRPT	2-FNLE	HST
0	0.40 (0.10)		1.59 (1.18)	1.60 (1.02)	0.50 (0.29)	0.20 (0.18)	
4	0.72 (0.65)		1.76 (1.54)	4.20 (1.67)	0.30 (0.25)		
8	0.76 (0.47)	0.10 (0.18)	2.25 (1.32)	3.90 (2.34)	0.50 (0.36)	0.24 (0.16)	
12	0.91 (0.56)	0.40 (0.10)	2.98 (1.19)	5.10 (1.64)	1.14 (0.15)	1.50 (0.15)	
16	1.09 (0.15)	0.90 (0.20)	2.12 (0.37)	4.90 (1.98)	0.80 (0.10)	0.50 (0.45)	
20	2.19 (2.06)	1.26 (0.27)	1.45 (1.31)	2.30 (1.78)	0.70 (0.40)	0.30 (0.12)	
23	2.37 (0.48)	1.80 (1.03)	1.74 (0.44)	2.70 (0.67)	0.90 (0.68)		

The values are expressed as mean (standard deviation), ($n = 6$).
PUT: Putrescine, CAD: Cadaverine, SPMD: Spermidine, SPM: Spermine, TRPT: Tryptamine, 2-FNLE: 2-phenylethylamine, HIS: Histamine. Agmatine, Serotonin and Tyramine were not detected.

Table 3
Biogenic amine contents (mg/kg muscle) in sea bass stored in cling film

Storage time (days)	PUT	CAD	SPMD	SPM	TRPT	2-FNLE	HST
0	0.40 (0.10)		1.59 (1.18)	1.60 (1.02)	0.50 (0.29)	0.20 (0.18)	
4	0.82 (0.35)	0.15 (0.10)	2.12 (0.17)	2.10 (0.89)	0.65 (0.38)	1.35 (1.01)	
8	0.94 (0.82)	0.32 (0.20)	1.92 (0.62)	1.95 (0.35)	0.26 (0.15)	0.24 (0.15)	
12	1.55 (0.40)	0.45 (0.12)	3.52 (1.50)	2.42 (1.24)	0.70 (0.64)		
16	3.87 (1.23)	1.50 (0.17)	3.20 (1.33)	2.81 (0.30)	1.20 (0.59)	0.40 (0.27)	
20	6.9 (1.32)	9.0 (2.05)	5.4 (1.17)	3.20 (1.49)	2.12 (1.24)	1.22 (0.16)	3.50 (1.82)

The values are expressed as mean (standard deviation), ($n = 6$).
PUT: Putrescine, CAD: Cadaverine, SPMD: Spermidine, SPM: Spermine, TRPT: Tryptamine, 2-FNLE: 2-phenylethylamine, HIS: Histamine. Agmatine, Serotonin and Tyramine were not detected.

Table 4
Biogenic amine contents (mg/kg muscle) in sea bass stored in aluminium foil

Storage time (days)	PUT	CAD	SPMD	SPM	TRPT	2-FNLE	HST
0	0.40 (0.10)		1.59 (1.18)	1.60 (1.02)	0.50 (0.29)	0.20 (0.18)	
4	0.56 (0.31)		1.52 (0.84)	2.54 (1.48)	0.61 (0.49)	0.32 (0.15)	
8	0.90 (0.17)	0.23 (0.29)	2.78 (1.37)	3.06 (1.01)	0.82 (0.48)	1.10 (0.80)	
12	1.49 (0.15)	0.56 (0.24)	3.14 (1.96)	2.02 (0.93)	1.20 (0.86)	1.06 (0.18)	0.82 (0.52)
16	1.86 (0.94)	1.60 (0.81)	1.28 (0.73)	1.90 (0.48)	0.32 (0.25)		
20	5.54 (1.94)	6.42 (1.29)	3.15 (2.34)	2.04 (0.32)	2.81 (0.32)	2.10 (0.86)	4.30 (1.50)

The values are expressed as mean (standard deviation), ($n = 6$).
PUT: Putrescine, CAD: Cadaverine, SPMD: Spermidine, SPM: Spermine, TRPT: Tryptamine, 2-FNLE: 2-phenylethylamine, HIS: Histamine. Agmatine, Serotonin and Tyramine were not detected.

during storage of sea bass in ice. Putrescine and cadaverine concentration increased with low levels during storage. Spermidine, spermine and tryptamine, 2-phenylethylamine content fluctuated throughout storage. Paleologos, Savvaidis, and Kontominas (2004) reported that histamine was not found in whole sea bass and gutted sea bass. However, histamine (2.7 mg/kg) was only found in cultured sea bass fillets. Poli et al. (2001) found that the concentration of putrescine, cadaverine, histamine, spermine and spermidine in reared sea bass was 4, 2, 5.1, 7.7 and 19 mg/kg, respectively, when the fish was 6 days in ice (1 °C). However,

when the fish were unfit for consumption, putrescine content was found to be 5 mg/kg and cadaverine, histamine, spermine and spermidine were not detected.

The concentrations of the biogenic amines present in the muscle of sea bass held in cling film are given in Tables 3. Putrescine and cadaverine were the major amines detected in sea bass wrapped in cling film. The putrescine and cadaverine contents increased throughout storage to about 6.9 and 9 mg/kg, respectively. Histamine was only detected on the 20th day of storage. Spermine, spermidine, tryptamine and 2-phenylethylamine all fluctuated during the storage period. Generally, biogenic amine accumulation in sea bass stored in WCF and WAF is higher than sea bass stored in ice. This might be due to the fact that lower storage temperature inhibits the formation of biogenic amines. Sensory analysis in the first part of the study revealed that sea bass stored in the four different conditions were still acceptable at 4 days for fish stored at 4 °C, 16 days for in ice storage, 8 days for WCF and WAF. At this time, formation of the important biogenic amines, which are histamine, putrescine and cadaverine, were less than 3.5 mg/kg during the whole storage period. Biogenic amines are produced by bacterial decarboxylation of amino acid. Therefore, once bacterial spoilage began, biogenic amines concentration tended to increase especially histamine, cadaverine, and putrescine.

The changes in biogenic amine contents in the muscle of sea bass in aluminium foil are presented in Tables 4. The level of cadaverine and putrescine rose during storage and putrescine and their levels increased to 5.5 and 6.4 mg/kg, respectively. The most important amine is histamine which was only detected on day 12 and day 20 with concentrations of 0.82 and 4.3 mg/kg muscle. At the time of rejection, the content of histamine in sea bass for WAF was 0.8 mg/kg. The level of biogenic amines was considerably higher in sea bass stored for WCF and WAF when compared with the iced storage conditions. This indicates that packing of fish in aluminium foil is not helpful to prevent accumulation of biogenic amines.

Total viable counts (TVC) for sea bass was 8.7 log10 cfu/ml for fish stored in ice, 8.7 log10 cfu/ml for fish stored at chill temperature, 9.6 log10 cfu/ml for fish wrapped in aluminium foil and 8.7 log10 cfu/ml for fish wrapped in cling film during last day of the storage. At this time histamine, cadaverine and putrescine contents were 3.5, 9 and 6.9 mg/kg for WCF storage conditions and 4.3, 6.4 and 5.5 mg/kg for WAF storage conditions, respectively. There is an inverse relationship between bacterial load and amine production since cling film is a type of MAP due to modification of atmosphere within cling film by decreasing O_2 concentration while increasing the content of CO_2. The cling film in-pack environment would, therefore, inhibit the further growth of aerobic bacteria, but aluminium foil could not as there was penetration of oxygen from outside into the unsealed aluminium foil pack.

Histamine concentration was only detected towards the end of storage for WCF and WAF storage conditions. Thus, there was not good correlation in histamine concentration among the storage conditions with sensory analysis. Therefore, the usefulness of the histamine content as an index of decomposition was not shown by the data obtained in this study. Sato, Okuzumi, and Fujii (1995) concluded that histamine and other biogenic amines were not reliable indices of spoilage in the case of common mackerel. However Nagayama et al. (1985) reported that histamine was useful as a quality index of scombroid fish. Cadaverine and putrescine content increased slowly with storage time but did not correlate with the sensory analysis. Therefore, they can not be used as a freshness index or degree of the spoilage. However, Fernandez-Salguero and Mackie (1987) indicated that cadaverine and putrescine show a steady rise once bacterial spoilage begins, hence, these amines are considered as potential indicators of fish quality. Dawood et al. (1988) also indicated that cadaverine and putrescine levels are potential indicators of fish quality.

Inhibition of enzymatic activity of food or bacterial decarboxylase activity and prevention of bacterial growth are very vital to control amine production. The most important factor affecting the production of biogenic amines is storage temperature (Klausen & Lund, 1986; Mackie et al., 1997; Richie & Mackie, 1980; Veciana-Nogues, Albala-Hurtado, Marine-Font, & Vidal-Carou, 1996). The easiest method of prevention of amine accumulation is rapid chilling of harvested fish and maintenance of low temperature until the point of consumption as found in this study for ice conditions. Hygienic handling of fish from the moment of capture to the point of consumption is also crucial to reduce the formation of biogenic amines.

4. Conclusion

Nucleotide degradation ratios (K and related values) results show that they can be successfully used as a freshness index except sea bass stored without ice. K, Ki and G value are also superior to H value because of better linear relationship between these indices and storage of fish. The biogenic amine contents generally increased with storage time. The most hazardous amine is histamine, detected only towards the end of the storage period for WAF and WCF storage conditions. Wrapping fish in cling film and aluminium foil is not advantageous in terms of nucleotide degradation product or biogenic amine formation.

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