

# Sensory and biochemical aspects of quality of whole bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres

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## Abstract

The quality of whole ungutted bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres containing either of two gas mixes (60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> and 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>) was evaluated by biochemical and sensory changes. At day 33 of storage, none of the lots were rejected on the basis of the target biochemical indices (pH, trimethylamine nitrogen (TMA-N), total volatile base nitrogen (TVB-N) and histamine) or the tasting panel scores. However, the control and the controlled atmosphere-stored lots were rejected upon inspection at 13 and 22 days, respectively. Scores for the various target biochemical parameters were highest ( $p < 0.05$ ) in the control, followed by the lot stored in gas mix two, which had the lower CO<sub>2</sub> concentration. There were changes ( $p < 0.05$ ) in the levels of biogenic amines (tyramine, histamine, cadaverine and agmatine) in the course of storage, but pH, TMA-N and TVB-N remained constant. Histamine levels exceeded 100 µg/g for the control fish and in gas mix one towards the end of storage. Histamine, cadaverine and agmatine could be used as quality indices for tuna stored in ice and atmospheres. Cadaverine and agmatine could also be used as freshness indices.

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**Keywords:** Tuna; Scombroid poisoning; Biogenic amines; Controlled atmosphere storage; Quality

## 1. Introduction

Tuna catches are of great commercial value worldwide, both fresh and canned. The demand for fresh product has been growing in recent years, all over the world, including Spain. Various tuna species abound on the coasts of the Canary Islands close to Spain, and transportation to the mainland requires costly air freighting for speed. The use of protective atmospheres for bulk whole tuna on board ships could help maintain the quality and prolong the shelf life of these species enough to make sea transport, which is more economical, viable.

There have been studies of storage of tuna in various atmospheres (López-Gálvez, De la Hoz, & Ordóñez, 1995; Oka, Fukunaga, Ito, & Takama, 1993; Tanaka, Nishino, Satomi, Yokoyama, & Ishida, 1996). However, these only concerned tuna steaks or fillets kept in modified atmospheres where the gas compositions are not corrected in the course of storage and they also do not concern, whole tuna using controlled atmospheres. Controlled atmospheres have proven to be more effective since the concentrations of gases are monitored and regulated throughout the storage.

Fish muscle undergoes a series of changes during storage under atmospheres and conventional chilled storage, resulting in its deterioration and loss of quality. In the first period of storage, the changes cause a loss of freshness and in the last period they produce spoilage. These changes have traditionally been assessed using indices such as nucleotides, ATP and breakdown compounds, total volatile base

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nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), ethanol or biogenic amines (Gill, 1990; Gill, Thompson, Gould, & Sherwood, 1987; Hebard, Flick, & Martin, 1982; Huss, 1995; Mackie, Pirie, & Yamanaka, 1997; Miet & Karmas, 1977; Ruiz-Capillas & Moral, 2001a, 2001b; Yamanaka, 1990). Biogenic amines are also associated with scombroid poisoning from consumption of spoiled tuna.

The objective of this work was to examine the quality of whole bigeye tuna, bulk-stored in controlled atmospheres from the time of capture upto the analysis of biochemical and sensory changes related to freshness and spoilage occurring in the muscle of this tuna.

## 2. Materials and methods

### 2.1. Sample preparation and procedure

The species used in this study was bigeye tuna (*Thunnus obesus*) caught in the Atlantic, near the Canary Islands. On board, the tunas were selected by sizes and cooled by immersion in melting water of ice. Later on, the tunas were placed in polyurethane boxes with plenty of ice until the boat reached port. Transport to the research centre, (Instituto del Frío [IF], Madrid, Spain) was by air and the tunas reached the IF with a maximum post-catching time of five days. In the IF, the batch of 180 kg of bigeye tuna, without gutting, had an average weight of  $5.39 \pm 1.2$  kg and an average length of  $67 \pm 5.4$  cm. They were washed, placed in boxes with ice, and divided into three lots. Lot 1 and lot 2 were kept in two hermetically-sealed stainless steel containers, containing gas mixtures: 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> and 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>, respectively, taken from pressurized bottles. The gas applied in the kind of controlled atmospheres, and the control of gas mixture was made following the procedure of Ruiz-Capillas and Moral (2001c). The containers and the control lot (T) stored in air were also kept in a chamber at  $2 \pm 1$  °C. All the lots were kept in these conditions until spoilage and they were analysed periodically. The temperature was recorded every 6 h to the end of the experiment and it was found to be within the proper limits of  $2 \pm 1$  °C for this species, without any abuse of temperatures (I.I.F., 1979).

### 2.2. Measurement of gases

The concentrations of CO<sub>2</sub> and O<sub>2</sub> in the gases were measured on an ABISS PACK 12 apparatus (Montpellier, France) every 24 h in the two containers. Gas samples (50 ml) for analysis using a gas-tight syringe (ABISS, Montpellier, France) were obtained through

the orifice of the containers, as described by Ruiz-Capillas and Moral (2001c).

### 2.3. Chemical analyses

Five fish were taken from each lot and they were gutted, skinned and cut into slices and then all the muscle was mixed to prepared a homogeneous representative samples for the chemical tests. The pH was determined in a Radiometer model PHM 93 pH-meter (Meterlab, Copenhagen, Denmark) at room temperature on homogenates in water in a ratio 1:10 (w/v) by the AOAC method (1995). TMA-N was determined according the AOAC method (1995). The TMA was extracted with toluene and this extract was reacted with picric acid, which interacted with the primary and secondary amines to produce a coloured reaction product (yellow picrates) with maximum absorption at 410 nm. TVB-N was determined according to the Antonacopoulos and Vyncke (1989) method. Determinations of biogenic amines (tyramine, histidine, putrescine, cadaverine, spermidine and agmatine) were carried out by extraction of a 25 g sample with 50 ml of 7.5% trichloroacetic acid using an Ultraturrax homogenizer (IKA-Werk, Janke & Kunkel, Staufen, Germany) (4000 g for 3 min) followed by centrifugation at 1500 g for 15 min at low temperature (4 °C). The supernatants were filtered through a 0.45 µm Millipore filter, and 10 µl of this filtrate was injected into HPLC model 1022 with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, CA) following the procedure described by Ruiz-Capillas and Moral (2001d).

### 2.4. Sensory evaluation

Two forms of sensory analysis were considered: inspection and taste panel. Inspection was performed by five trained persons with five samples of tuna prior to the other analysis. Each sample of the lot was classified using an analysis of anatomical parameters (Table 1). Each characteristic was scored on a scale of 0–10, with 10 representing fresh fish and 0 putrefied fish and lots scoring less than four were rejected. The taste panels were panels of seven semi-trained tasters selected from Instituto del Frío (Madrid, Spain) personnel. The samples had been cut into cubes, covered and placed in a microwave oven for 6 min. These were presented to the panel members who assessed the different parameters (Table 2). Points were given on a scale of 0–10, and lots scoring less than four were rejected. The sensory analyses were carried out following the scheme described by Ruiz-Capillas and Moral (2001a, 2001c).

Table 1

Sensorial analysis: raw assessment of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>(C2) in bulk storage until spoilage<sup>a</sup>

Parameters	Lots	Days of storage					
		0	5	11	18	25	33
General appearance	T	10a1	9.6a1	5.0a2	1.8a3	1.0a4	
	C1		9.6a1	5.0a2	6.6b2	5.3b2	2.4a3
	C2		10a1	4.0a2	7.8b2	4.5b2	1.4b3
Gill colour	T	9.8a1	8.6a2	4.2a3	1.3a4	3.4a4	
	C1		8.6a2	6.5a3	4.5b3	5.5b3	4.2a3
	C2		10a1	6.5a2	5.9b2	4.5b2	2.2b3
Odour	T	9.7a1	8.4a2	6.4a3	1.0a4	0.5a5	
	C1		9.4a1	6.4a2	4.3b2	2.0a3	3.5a2
	C2		10a1	3.6a2	4.8b2	3.2a2	2.1b3
Eyes	T	9.8a1	9.2a1	7.0a2	2.8a3	1.0a4	
	C1		9.2a1	7.0a2	5.2b3	2.4b4	2.5a4
	C2		10a1	5.9a2	3.5b3	2.0b4	2.5a4
Meat colour	T	9.8a1	8.3a2	7.0a2	0.7a3	0.0a3	
	C1		7.9a2	7.0a2	7.4b2	3.1b3	2.5a3
	C2		9.5a1	7.0a2	6.2b2	2.5b3	2.5a3

Each value is the mean score of five samples of bigeye tuna evaluated by five trained people, with a scale between 0 and 10 (10 excellent and 0 very bad). The reject point was four points. Different letters in the same column and different numbers in the same row indicate significant differences ( $P < 0.05$ ).

<sup>a</sup>T, control lot, stored in air throughout the experiment.

Table 2

Sensorial analysis: cooked assessment of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>(C2) in bulk storage until spoilage<sup>a</sup>

Parameters	Lots	Days of storage				
		0	5	11	18	25
General appearance	T	9.9a1	6.8a2	8.1a2	6.8a2	
	C1		7.3a2	7.0a2	6.7a2	7.5a2
	C2		6.3a2	7.5a2	7.0a2	7.9a2
Colour	T	8.7a1	6.5a2	7.8a2	6.2a2	
	C1		6.6a2	6.8a2	6.3a2	5.8a2
	C2		6.1a2	6.9a2	6.9a2	6.0a2
Odour	T	8.5a1	5.5a2	5.2a2	5.4a2	
	C1		5.9a2	5.4a2	5.1a2	4.6a2
	C2		5.2a2	5.4a2	4.8a2	4.4a2
Flavour	T	9.4a1	7.4a2	6.4a2	5.6a3	
	C1		7.6a2	7.0a2	7.1a2	7.2a2
	C2		6.9a2	7.6a2	4.6b3	6.4b2
Firmness	T	9.1a1	6.9a2	5.8a2	6.3a2	
	C1		7.1a2	5.9a2	6.5a2	6.9a2
	C2		6.6a2	5.9a2	6.3a2	7.2a2
Overall Acceptability	T	9.5a1	6.4a2	5.4a2	6.1a2	
	C1		6.7a2	6.7a2	7.2a2	5.2a2
	C2		5.7a2	6.7a2	5.1a2	5.5a2

Each value is the mean score of five samples of bigeye tuna evaluated by seven semi-trained taste panelists, with a scale between 0 and 10 (10 excellent and 0 very bad). The reject point was four points. Different letters in the same column and different numbers in the same row indicate significant differences ( $P < 0.05$ ).

<sup>a</sup>T, control lot, stored in air throughout the experiment.

## 2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out for the different samples. The mean differences be-

tween pairs were resolved by means of confidence intervals using a least significance range test at  $p \leq 0.05$ . The computer programme used was Statgraphics (STATC Inc., Rockville, MD).

### 3. Results and discussion

#### 3.1. Measurement of gases

Measurements of the atmospheres for containers 1: 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) and 2: 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub> (C2) are shown in Fig. 1. The atmosphere inside the containers did not stabilize until after 6–7 days with daily gas sweeps of 1 h, despite the fact that the averaged concentrations of both CO<sub>2</sub> and O<sub>2</sub> are close to the mixture of injected gas (50–55% CO<sub>2</sub> and 38–40% CO<sub>2</sub>, in the C1 and C2, respectively), immediately after the sweep. The reason may be that the individual fish were very large, with tougher skin than hake, in which atmospheres stabilize much more quickly (Ruiz-Capillas & Moral, 2001c). However, in the present case, as with hake, the O<sub>2</sub> concentration stabilized much more readily in container 1 where the level was lower, than in container 2 where over 40% was injected.

#### 3.2. pH

The results for pH are shown in Fig. 2. Initial pH values were low (5.4), and although they increased during storage, the final levels did not exceed six in any of the lots. Wei, Chen, Kourber, Otwell, and Marshall (1990) observed similar pH levels and behaviour in ice-stored tuna. The increases in pH are related to the ac-

cumulation of basic substances such as ammonia and TMA, produced during fish muscle spoilage (Hebard et al., 1982). Some authors (Huss, 1995; Moral, 1987) have set the level of acceptance at about pH 7. If we apply this limit, the quality of the tuna was acceptable throughout the present experiment.

#### 3.3. Trimethylamine nitrogen (TMA-N)

Fig. 3 shows the TMA-N levels determined in tuna. Initial levels were very low, reflecting the freshness of the tuna. Levels increased notably from day 11 until the end of storage, except for lot C2 in which differences were observed from day 18. Differences ( $p < 0.05$ ) between the control and the atmosphere-stored lots were not detected until day 25 of storage. TMA-N levels were highest in the control and fish stored in gas mix 1, followed by the lot stored in gas mix C2 (Fig. 3). However, such differences in evolution of the various lots are of no great importance given the very low final TMA-N levels; in no case did these exceed 2 mg/100 g, as compared to a legal limit of 12 mg/100 g (Ruiz-Capillas & Moral, 2001a). Comparable TMA-N levels have been reported in ice-stored albacore tuna (Perez-Villareal & Pozo, 1990). The results for TMA-N are consistent with those for pH, confirming that, after 33 days of storage under the given conditions, the tuna were perfectly fit for consumption.

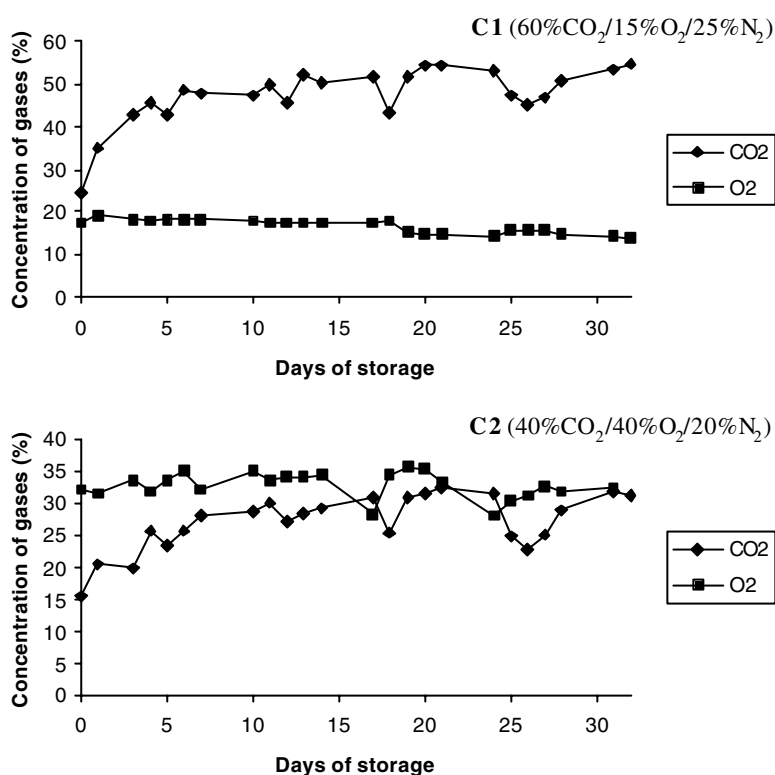


Fig. 1. Concentration of CO<sub>2</sub> and O<sub>2</sub> in the container of lot C1 and C2 stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub> (C2), respectively.

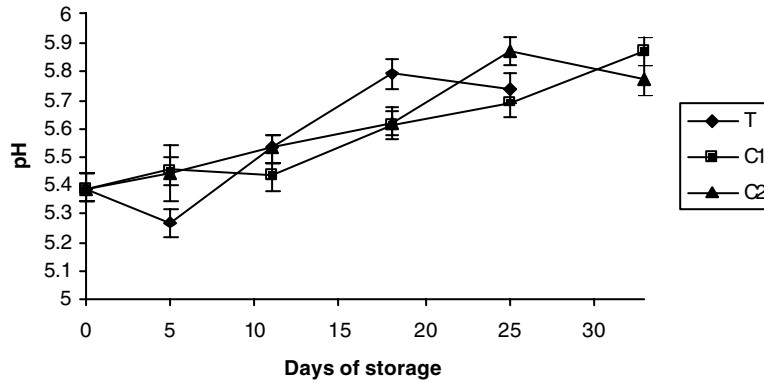


Fig. 2. pH of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub> (C2) in bulk storage until spoilage. T, control lot, stored in air throughout the experiment.

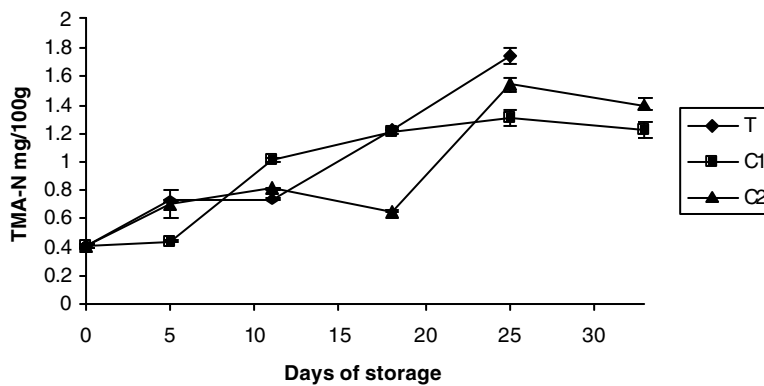


Fig. 3. Trimethylamine nitrogen (TMA-N) (mg/100 g) of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub> (C2) in bulk storage until spoilage. T, control lot, stored in air throughout the experiment.

#### 3.4. Total volatile base nitrogen TVB-N

Initial TVB-N levels in the tuna were very high (Fig. 4) when compared to the initial levels of TMA-N and pH. They were also high given that the legal limit for this index is 30 mg/100 g (Ruiz-Capillas & Moral,

2001a). Nevertheless, Gallardo, Perez-Martin, Franco, Aubourg, and Sotelo (1990) and Watanabe, Yamanaka, and Yamanakawa (1992) reported very similar initial TVB-N concentrations in albacore tuna. TVB-N concentrations remained practically unchanged in the various lots throughout storage. Levels were higher

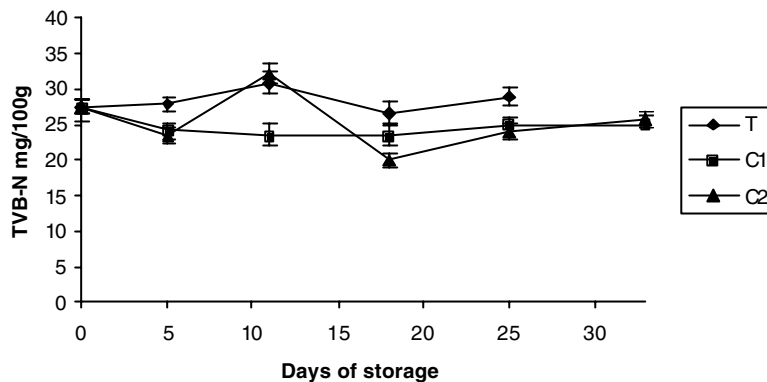


Fig. 4. Total volatile base nitrogen (TBV-N) (mg/100 g) of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub> (C2) in bulk storage until spoilage. T, control lot, stored in air throughout the experiment.

( $p < 0.05$ ) in the control than in the atmosphere-stored lots. There were not significant differences between the atmosphere lots at the end of storage (days 25 and 33), whereas, the level of TMA-N was higher in the lot stored in gas mix 2 (Fig. 3). Watanabe et al. (1992) also observed a slow rate of increase of TVB-N in albacore. The increases of TVB-N reported by other authors during storage of various fish species in ice (Hebard et al., 1982; Huss, 1995) have been associated with microbiological deterioration of these species and the production of spoilage substances, such as the volatile amine TMA and ammonia. This suggests that there was hardly any microbial deterioration in bigeye tuna stored under the experimental conditions. Species such as bigeye have very tough skin and transcutaneous microbial contamination takes longer, so that autolytic deterioration will be more prolonged than in other fish species. The absence of such increases in the present experiment therefore suggests that both the control and the atmosphere lots retained good quality throughout the storage period.

### 3.5. Sensory evaluation: Inspection and tasting

In sensory analysis of raw tuna (Table 1), the control was rejected at day 13 of storage, while the lots stored in atmospheres were rejected at day 22. Significant ( $p < 0.05$ ) differences between the atmosphere-stored lots and the control were first detected at day 18. There were differences between the atmosphere-stored lots in terms of general appearance, gill colour and odour at the end of storage (day 33), with lot C2 (gas mix 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>) scoring lower for these three parameters. This lot also presented higher levels of the biochemical parameters. However, these inspections did not clearly relate to the biochemical indices, since no lots were rejected on the basis of biochemical indices. Again, there was no apparent correlation between the results of inspection of raw tuna and the taste panel scores. The panel (Table 2) did not reject any of the lots and detected no differences ( $p < 0.05$ ) between the control and the atmosphere-stored lots before day 18 of storage. At day 25 of storage, the panel detected differences between atmosphere-stored lots only in flavour, lot C2 again scoring lower. On the other hand, the panels analysis did relate to the biochemical parameters.

### 3.6. Biogenic amines

Fig. 5 shows the evolution of biogenic amines in atmosphere-stored tuna. Initial concentration was highest for spermidine (21.8 µg/g), followed by histamine (7) and putrescine (4.5 µg/g). Both amines perform important physiological functions in the living fish (Smith, 1980). Concentrations of other amines were all below 3 µg/g initially.

The evolutions of spermidine and putrescine during storage showed the same trend, with fluctuations in all three lots (Fig. 5). However, other authors have reported significant increases in putrescine concentrations in the course of storage in tuna and other species such as herring and hake (Ababouch, Afilal, Benabdeljelil, & Busta, 1991; López-Gálvez et al., 1995; Özogul, Taylor, Quantick, & Ozogul, 2002; Ruiz-Capillas & Moral, 2001d).

Increased concentrations of histamine ( $p < 0.05$ ) were detected after day 11 of storage. Levels were highest in the control, followed by the lot stored in gas mix 2 (lower CO<sub>2</sub> concentration). At the end of storage, lot C2 presented histamine levels of 78.1 µg/g, that is similar to the level reached in the control at day 25 (Fig. 5). In lot C1, final histamine levels did not reach 100 µg/g or more (Fig. 5). The concentration regarded by the EC (1996) as hazardous (by histamine) is 100–200 µg/g for this species of fish. This tallies with the results for other biochemical indices, which again did not reach established limits. In the case of histamine, however, there were significant ( $p < 0.05$ ) changes in histamine levels during storage, which did not follow the pattern for conventional indices such as TMA-N and TVB-N. The changes in histamine were consistent with the changes observed in the inspection of raw tuna (Table 1). Histamine increases proportionally as freshness decreases, and it could therefore be used to replace the traditional quality indices for bulk tuna stored either in ice or atmospheres. Other authors (Özogul et al., 2002; Taylor, 1986) have also suggested that histamine could be a good quality index for tuna stored in ice.

Tyramine behaved in a similar way to histamine. There were significant ( $p < 0.05$ ) changes in the three lots after day 11 of storage, but there were no significant differences between the atmosphere-stored lots (Fig. 5).

The changes observed in agmatine and cadaverine during storage were very similar (Fig. 5). For the control, concentrations increased ( $p < 0.05$ ) from the outset of storage, while, in the atmosphere lots, these concentrations began to increase slightly as from day 5 to day 18 (Fig. 5). Agmatine and cadaverine began to increase earlier than histamine and tyramine. As in the case of histamine, significant differences ( $p < 0.05$ ) in the agmatine and cadaverine levels were detected between the different atmosphere-stored fish after day 11 (Fig. 5). The final concentrations of these two amines in the control were very high (117.6 for agmatine and 93.2 µg/g for cadaverine) at day 25 of storage – indeed higher than the observed histamine concentrations. Other authors (López-Gálvez et al., 1995) have also reported high concentrations of these two amines in albacore (*Thunnus alalunga*). The early onset of changes in the levels of agmatine and cadaverine could be due to the autolytic changes occurring in fish muscle prior to microbial growth (Hebard et al., 1982; Ruiz-Capillas & Moral,

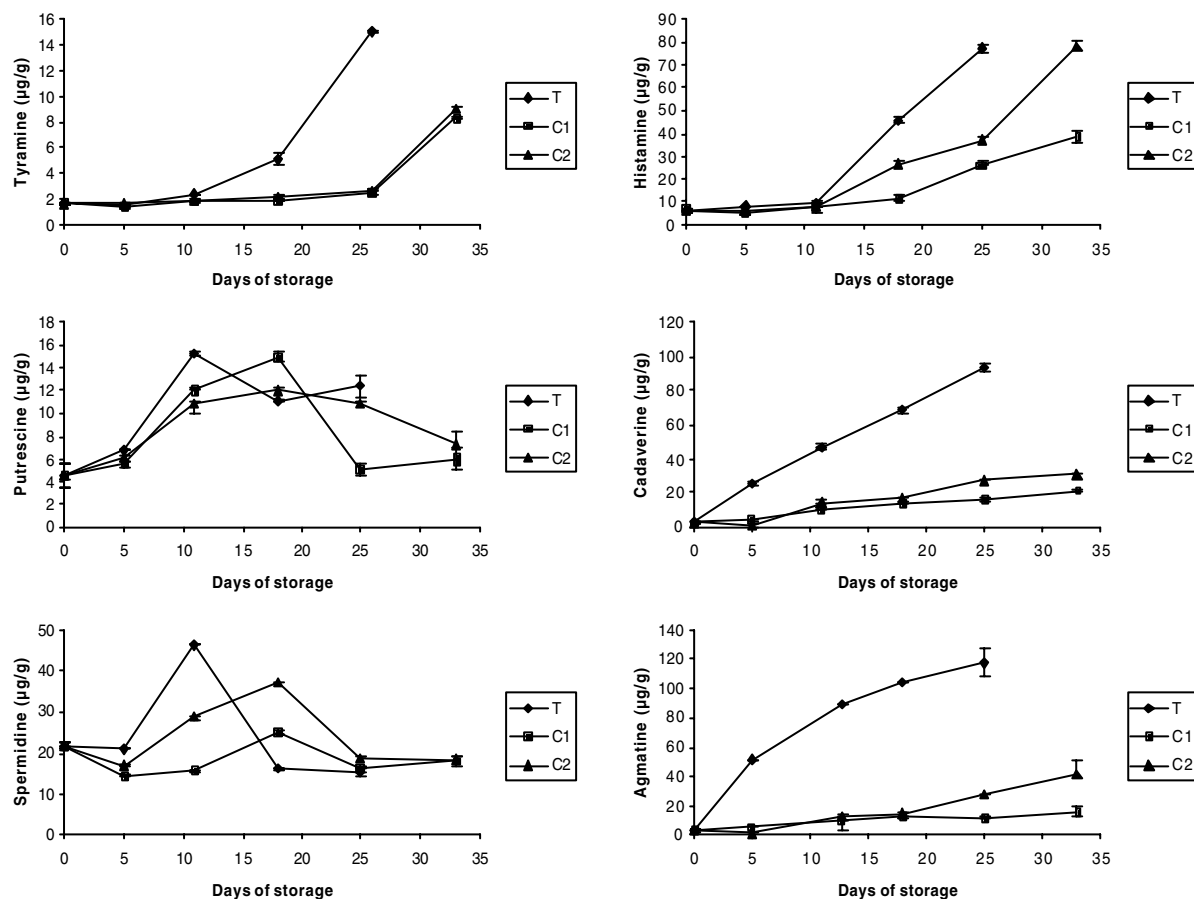


Fig. 5. Biogenic amines (tyramine, histamine, putrescine, cadaverine, spermidine and agmatine) ( $\mu\text{g/g}$ ) of chilled bigeye tuna (*Thunnus obesus*) stored under controlled atmosphere having a gas mixture of 60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub> (C1) or 40%CO<sub>2</sub>/40%O<sub>2</sub>/20%N<sub>2</sub>(C2) in bulk storage until spoilage. T, control lot, stored in air throughout the experiment.

2001d). Moreover, such autolytic changes in this species are more prolonged than in other species, such as hake, because the thickness of the skin slows down transcutaneous microbial contamination. These amines could be suitable as indices of freshness. We made similar observations in our study of hake stored in ice and controlled atmospheres (Ruiz-Capillas & Moral, 2001d). For tuna, these indices could be used instead of the conventional TMA-N or TVB-N indices, which, furnish hardly any information on changes during spoilage.

In conclusion, the control and the controlled atmosphere tuna lots retained quality up to 13 days and 22 days, respectively. However, the more effective gas mix for bulk storage of tuna is gas mix 1 (60%CO<sub>2</sub>/15%O<sub>2</sub>/25%N<sub>2</sub>). The conventional quality indices, pH, TMA-N and TVB-N, did not adequately reflect the changes that occur in bigeye tuna stored in ice or controlled atmospheres. These indices could be replaced by the biogenic amines of histamine, cadaverine and agmatine. Histamine could be an effective index of spoilage, while cadaverine and agmatine could be good indices of freshness.

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