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Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres

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Summary. This paper analyses the presence of and changes in free amino acids and biogenic amines in red and white muscle of bigeye tuna during storage in controlled atmospheres with 2 gas mixes containing different concentrations of CO_2 and O_2. Levels of amines were generally higher in white than in red muscle, with the exception of putrescine and spermidine. Levels of biogenic amines increased ($p < 0.05$) throughout storage, commencing later in red than in white muscle. A correlation between the amino acid histidine and the biogenic amine histamine was observed, but only in white muscle. Only in the case of tryptophan did white and red muscle differ ($p < 0.05$) in terms of essential free amino acid content. They also differed in anserine content. Concentrations of the non-essential FAAs glutamic acid, glycine and alanine were higher in red than in white muscle. The effectiveness of the atmospheres was reflected in the evolution of both biogenic amines and FAAs. Gas mix 1, containing a higher concentration of CO_2, was the more effective.

Keywords: Free amino acids – Histidine – Red and white muscle – Tuna – Controlled atmosphere

Introduction

The effectiveness of protective atmospheres in prolonging the shelf life and maintaining the quality of fish has been demonstrated in many species (Stammen, 1990; Reddy et al., 1992; López-Galvez et al., 1995; Ruiz-Capillas and Moral, 2001a; Ruiz-Capillas et al., 2001; Sivertsvik et al., 2002). However, although the autolytic and microbial processes that take place in fish muscle during storage are slowed down, they are not halted and hence the fish eventually spoils. These processes affect muscle compounds such as proteins and free amino acids (FAAs). There have been studies of the evolution of free amino acids in the course of fish storage (Sakaguchi et al., 1982; Murata and Salagucki, 1986; Ochiai et al., 1990; Mendes et al., 1999; Ruiz-Capillas and Moral, 2001b). The changes in these amino acids have been linked to the flavour of the fish (Hayshi et al., 1981; Konosu and Yamaguchi, 1982; Yamanaka and Shimada, 1996) and also to the technological processes used (Pérez-Martin et al., 1988; Chang et al., 1992). Also, FAAs have been used as quality indices in various fish species under different storage conditions (Matsumoto and Yamanaka, 1990; Otsuka et al., 1992; Chang et al., 1992). Also, FAAs have been used as quality indices in various fish species under different storage conditions (Matsumoto and Yamanaka, 1990; Otsuka et al., 1992; Yamanaka and Shimada, 1996; Ruiz-Capillas and Moral, 2001b). FAAs can be very useful as quality indices, since they start to undergo changes when the fish dies. Investigation of FAAs will therefore provide information on the automatic and microbial changes undergone by fish muscle in the process of spoilage. In fish species such as tuna, where automatic spoilage is more prolonged than in other species, the changes in free amino acids are presumably more pronounced and hence all the more deserving of investigation. FAAs are formed in fish as a result of muscle proteolysis. As muscle spoilage progresses, these FAAs serve as a substrate for microbial growth, giving rise to a variety of products including biogenic amines. There has been considerable research into these amines in tuna, particularly histamine (Lopez-Galvez et al., 1995; Silva et al., 1998; Ben-Gigerei et al., 1999; Kanki et al., 2000; Kim et al., 2002; Du et al., 2002) which has been associated with scombrid poisoning. The FDA (1990) has set the toxic threshold for histamine at 100 mg/kg. Biogenic amines have also been used as quality indices in these species; (Miet and Karmas, 1977; Veciana-Nogues et al., 1998; Mendez et al., 1999; Rossi et al., 2002; Ohashi, 2002) however, it is

important to note that tuna contains varying proportions of red and white muscle, which perform different physiological functions in the live animal (Huss, 1995). There are also many differences in the chemical composition of the two muscle types. This means that the components of the two muscle types will not undergo the same chemical changes during storage in different atmospheric and refrigeration conditions.

The object of this study was to assess the changes occurring in free amino acids and biogenic amines in dark and ordinary muscle of bigeye tuna during storage in controlled atmospheres containing gas mixes with different concentrations of CO_2 and O_2.

Materials and methods

Sample preparation and procedure

The bigeye tuna (*Thunnus obesus*) analysed in this study was caught in the Atlantic water, near the Canarian Islands. On board, the tunas were selected by sizes, cooled by inmersion in melting water of ice and placed in poliuretane boxes with plenty of ices until the boat reached port. The transport until the research centre, (Instituto del Frio, Madrid, Spain) was via air way and the tunas reached the IF with a maximum post-catching time of 5 days. In the IF, the bacht of 180 kg of big eye tuna without gutted had an average weight of 5.39 ± 1.2 kg and an average size of 67 ± 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 hermetic stainless steel containers, with a two gases mixture: 1 ($60\%CO_2$: $15\%O_2$: $25\%N_2$) and 2 ($40\%CO_2$: $40\%O_2$: $20\%N_2$) respectively, taken from pressurized bottles. The gas applied in the kind of controlled atmospheres, and the instauration and control of gas mixture was made following the procedure of Ruiz-Capillas and Moral (2001a). The containers and the control lot (T) were also kept in a chamber at 2 ± 1°C. All the lots were kept in these conditions until their spoilage, and they were analysed periodically.

Analytical methods

For each lot, 5 bigeye tuna were withdraw at random to prepare a homogeneous representative sample. The tuna was cut into slices from which skin and bone were removed, then the muscles was separated independ: red (R) and white (W), to carry out the analysis which were normally run periodically and all determinations were performed in triplicate, obtaining the average of these analysis.

Determination of free amino acids (FAAs) and peptides. The sample was prepared in accordance with the technique described by Yamanaka (1989). The free amino acids were determined by using a high-preformance liquid chromatograph. (HPLC) model 1022 with a pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca (USA)) using a chromatography column of lithium cationic exchange and o-phthalaldehyde (OPA) as derivative reagent to form fluorescent derivatives with free aminoacids which were detected in a fluorescence spectrometer LC 240 (Perkin Elmer, Spain) at 330 nm excitation and 465 emission (Ruiz-Capillas and Moral, 2001b).

Determination of biogenic amines (tyramine, histidine, putrescine, cadaverine, and agmatine) was carried out by extraction of 25 g of sample with 50 mL of 7.5% trichloroacetic acid using an ultraturrax homogenizer (20000 r.p.m., 3 min) followed by centrifugation at 5000 rpm for 15 min at low temperature (4°C). The supernatants were filtrated through a 0.45 μm Millipore (HVLP) filter, and 10 μL of this filtrate were injected into a HPLC model 1022 with a pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca (USA)) and follow the methodology of Ruiz-Capillas and Moral (2001c).

Statistical analysis

Statistical analysis was performed by analysis using analyses of variance (ANOVA) one-way followed by a least significant difference (LSD) test at $p < 0.05$ using SPSS 6.0 (SPSS Inc., Chicago, Ile).

Results and discussion

Biogenic amines

The concentrations of biogenic amines in red and white muscle of tuna kept in controlled atmospheres are shown in Table 1. Initial concentrations of amines were generally higher ($p < 0.05$) in white than in red muscle. The exceptions were putrescine, levels of which were similar in both muscle types, and spermidine, levels of which were higher in red than in white muscle. Also, initial spermidine concentrations were much higher than those of all the other biogenic amines (21.8 and 42.5 mg/kg in white and red muscle respectively). Spermidine levels are higher for physiological reasons, given that spermidine performs vital functions in the live animal (Taylor, 1985; Smith, 1980; Halász et al., 1994). Other authors have reported similar levels of spermidine in tuna (Wendakoon et al., 1990; Watanabe et al., 1992; Lopez-Galvez et al., 1995; Du et al., 2002). Also, Wendakoon et al. (1990) found higher amine concentrations in red than in white muscle of herring.

Concentrations of all the studied amines except spermidine increased during storage (Table 1). The same tendency was reported by other authors (Lopez-Galvez et al., 1995; Wendakoon et al., 1990; Du et al., 2002). Such increases were observed from days 11–25 in the control, but not until days 25–33 in the atmosphere-stored lots. In both cases (red and white muscle), amine levels were higher ($p < 0.05$) in the control than in the atmosphere lots (Table 1). The atmosphere would therefore seem to be effective in that it retarded the biochemical and microbiological changes that occur in stored tuna muscle. Also, amine levels were higher in the lot stored in gas mix 1 (60% CO_2) than in gas mix 2 (40% CO_2), demonstrating that the former is the more effective. Other authors have likewise demonstrated the effectiveness of atmospheres in reducing biogenic amine concentrations in tuna, sardine, herring, hake and other species (Lopez-Galvez et al., 1995; Ruiz-Capillas and Moral 2001d; Ozogul et al., 2002).

Tyramine and putrescine presented the lowest concentrations of all the biogenic amines during storage (Table 1). As in the initial determinations, tyramine concentrations

Table 1. Biogenic amines (mg/kg) in red (R) and white (W) muscle of tuna stored in controlled atmosphere with the gas mixture (1) (60/15/25, $CO_2/O_2/N_2$%) and (2) (40/40/20, $CO_2/O_2/N_2$%) during of the storage until spoilage. T, control lot, stored in air throughout the experiment

Biogenic amines	Lots	Days of storage					
		0	5	11	18	25	33
Tyramine	WT	1.6 a/1	1.5 a/1	2.3 a/2	5.1 a/3	15.1 a/4	
	W1		1.4 a/1	1.8 a/1	1.9 b/1	2.5 b/2	8.3 a/3
	W2		1.6 a/1	1.9 a/1	2.1 b/1	2.6 b/2	9.1 b/3
	RT	0.5 b/1	1.3 a/2	2.6 a/2	3.8 c/3	8.2 c/4	
	R1		1.2 a/2	1.5 b/2	1.6 b/2	2.9 b/3	5.0 c/4
	R2		1.2 a/2	1.5 b/2	1.6 b/2	2.8 b/3	6.1 d/4
Histamine	WT	6.2 a/1	8.1 a/2	9.5 a/2	45.5 a/3	77.1 a/4	
	W1		5.2 b/1	7.8 b/2	11.5 b/3	26.8 b/4	38.4 a/5
	W2		5.9 b/1	7.9 b/2	26.5 b/3	37.2 c/4	78.1 b/5
	RT	2.0 b/1	4.7 b/2	5.4 c/2	7.5 c/3	17.0 d/4	
	R1		1.8 c/1	3.6 d/2	7.5 c/3	6.8 e/3	16.0 c/4
	R2		4.0 b/2	4.7 c/2	4.5 d/2	8.0 f/3	31.5 d/4
Putrescine	WT	4.5 a/1	6.8 a/1	15.2 a/1	11.1 a/1	12.4 a/1	
	W1		5.5 a/1	12.1 b/1	15.0 b/1	5.0 b/1	6.0 a/1
	W2		6.1 a/1	10.9 c/1	12.1 a/1	10.8 c/1	7.3 a/1
	RT	3.0 a/1	8.1 b/2	8.2 d/2	11.1 a/3	12.4 a/3	
	R1		1.8 c/1	7.8 d/2	5.9 c/2	8.2 d/2	8.5 c/2
	R2		7.9 b/2	6.8 d/2	7.5 d/2	12.9 a/3	9.3 d/1
Cadaverine	WT	2.4 a/1	25.8 a/2	47.2 a/3	68.9 a/4	93.2 a/5	
	W1		4.0 b/2	10.3 b/3	14.0 b/4	15.8 b/5	21.5 a/6
	W2		1.3 c/2	14.5 c/3	16.5 c/4	27.0 c/5	31.0 b/6
	RT	1.0 b/1	3.7 b/2	11.9 b/3	13.4 b/4	22.0 d/5	
	R1		0.8 c/1	3.5 d/2	4.5 d/3	5.8 eb/4	56.5 c/5
	R2		1.0 c/1	2.0 d/2	4.5 d/3	9.3 f/4	77.9 d/5
Agmatine	WT	3.1 a/1	51.8 a/2	89.3 a/3	103.8 a/4	117.6 a/5	
	W1		5.0 b/2	10.0 b/3	12.0 b/3	11.6 b/3	15.8 a/4
	W2		0.9 c/2	12.8 c/3	14.8 c/4	27.8 c/5	41.2 b/6
	RT	0.2 b/1	4.5 b/2	8.7 d/3	6.5 d/3	13.1 b/4	
	R1		0.63 c/1	3.5 e/2	4.8 e/3	6.0 d/3	12.5 c/4
	R2		0.78 c/1	2.3 e/2	2.0 f/2	4.5 d/3	22.1 d/4
Spermidine	WT	21.8 a/1	21.0 a/1	46.5 a/3	16.0 a/2	15.3 a/2	
	W1		14.5 b/2	15.7 b/2	25.0 b/3	16.1 a/2	18.0 a/2
	W2		17.0 b/2	29.1 c/3	37.1 c/3	18.7 a/2	18.3 a/2
	RT	42.5 b/1	69.8 c/2	45.7 a/1	24.5 b/3	37.1 b/4	
	R1		27.5 d/2	33.0 c/3	33.5 d/3	38.5 b/4	26.0 b/2
	R2		38.4 e/2	30.1 c/3	34.0 d/4	49.0 c/5	22.9 c/6

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$)

were higher in white than in red muscle throughout storage. Putrescine concentrations, on the other hand, were very similar in white and red muscle at the outset of storage and were higher in red than in white muscle by the end. The pattern for spermidine was similar, possibly because both are located on the same metabolic route (Halász et al., 1994). Wendakoon et al. (1990) also reported that putrescine levels were always much higher in the red muscle than that in the white muscle of tuna.

In the case of the other amines, concentrations did differ ($p < 0.05$) between white and red muscle in the course of storage (Table 1). In the case of histamine, pronounced increases were recorded at days 18–25 in white muscle but only on the last day of storage in the case of red muscle. Histamine has frequently been used as a quality index for tuna (Rossi et al., 2002; Ohashi, 2002). In the present case, the behaviour of histamine seems to show that spoilage commenced earlier in white than in red muscle. Nevertheless, the histamine level identified as toxic by the FDA (1990) (100 mg/kg) was not reached in either muscle type by the end of storage. Consumption of tuna containing histamine in excess of 100 mg/kg has been associated with symptoms of scombrid poisoning in the population (Taylor, 1985; Halász et al., 1994; Kanki et al., 2000). In this connection, the

present study tends to confirm that the experimental storage conditions can successfully maintain tuna quality.

In the case of cadaverine and agmatine, changes ($p < 0.05$) again occurred later in red than in white muscle (Table 1). However, concentrations of these amines at the end of storage were higher than those of histamine. The amine for which concentrations were highest at the end of storage was agmatine. At that time, cadaverine and agmatine concentrations in the white muscle control were 93.2 and 117.6 mg/kg respectively, that is, higher than the histamine concentrations, while the concentrations of both in red muscle were similar to the concentration of histamine (Table 1).

In the course of storage, agmatine and cadaverine concentrations changed earlier than concentrations of the other target amines (Table 1). Changes were observed in the atmosphere lots from the outset, as in the control, but these were less pronounced – possibly because spoilage was mitigated and retarded by the atmospheres. This is similar to our findings with histamine and tends to confirm the effectiveness of the atmospheres. Both agmatine and cadaverine have been associated with the autolytic changes responsible for loss of freshness that take place in fish muscle before the onset of microbial deterioration (Yamanaka, 1989; Ruiz-Capillas and Moral, 2001c). Note that these changes cannot readily be measured using traditional quality indices (Gill, 1990; Caill-Milly et al., 2001).

Biogenic amine-producing amino acids

Biogenic amines are produced by decarboxylation of the free amino acids in fish muscle (Smith, 1980; Halász et al., 1994). The free amino acid in highest concentrations

Table 2. Changes in FAA productive of biogenic amines (mg/100 g) levels in red (R) and white (W) muscle of tuna stored in controlled atmosphere with the gas mixture (1) (60/15/25, $CO_2/O_2/N_2$%) and (2) (40/40/20, $CO_2/O_2/N_2$%) during of the storage until spoilage. T, control lot, stored in air throughout the experiment

Free amino acids	Lots	Days of storage					
		0	5	11	18	25	33
Tyrosine	WT	1.77 a/1	5.78 a/2	3.75 a/3	5.67 a/2	7.10 a/3	
	W1		5.08 a/2	5.69 b/2	8.42 b/3	10.17 b/4	10.45 a/4
	W2		5.98 a/2	6.93 b/2	5.35 a/2	7.10 a/4	4.35 b/2
	RT	2.52 b/1	2.69 b/1	1.93 c/1	3.76 c/2	6.40 c/3	
	R1		1.77 b/1	2.15 c/1	3.50 c/2	5.50 c/3	11.38 a/4
	R2		2.34 b/1	3.44 c/2	4.00 c/2	5.78 c/3	6.89 c/1
Histidine	WT	118.71 a/1	152.01 a/2	158.35 a/2	132.9 a/2	108.07 a/1	
	W1		138.12 b/2	125.12 b/3	135.14 a/4	98.23 b/5	95.67 a/5
	W2		125.45 c/2	138.12 c/3	125.12 b/2	125.34 c/2	105.70 a/3
	RT	62.90 b/1	70.13 a/2	66.89 d/2	75.75 d/3	68.30 d/2	
	R1		53.20 b/2	68.25 d/3	68.42 e/3	63.98 d/1	70.03 c/3
	R2		55.21 b/2	75.50 e/4	70.82 e/4	70.05 d/4	71.32 c/4
Arginine	WT	1.25 a/1	4.70 a/2	5.15 a/2	5.10 a/2	4.69 a/2	
	W1		2.98 b/2	11.12 b/3	8.67 b/4	13.94 b/5	10.16 a/3
	W2		2.35 b/2	4.78 a/3	3.97 a/3	6.17 a/4	3.87 b/3
	RT	1.75 a/1	1.70 c/1	0.62 c/2	3.65 a/3	3.60 a/3	
	R1		0.50 c/2	0.59 c/2	1.80 c/1	1.80 c/1	8.70 a/3
	R2		0.70 c/2	1.88 c/1	2.25 c/1	2.25 c/1	3.14 b/3
Lysine	WT	18.13 a/1	58.12 a/2	17.32 a/1	44.23 a/3	14.25 a/1	
	W1		6.78 b/2	35.11 b/3	53.86 b/4	42.87 ba/5	43.52 a/5
	W2		13.12 c/2	44.57 c/3	15.24 c/2	22.65 c/4	14.65 b/2
	RT	38.10 b/1	38.25 d/1	18.09 a/2	22.70 d/3	15.60 a/2	
	R1		6.20 b/2	21.00 a/3	25.40 d/4	18.05 a/3	25.10 c/4
	R2		5.14 b/2	23.75 a/3	9.10 e/2	12.42 a/2	14.86 a/2
Ornithine	WT	1.45 a/1	5.36 a/2	0.96 a/1	1.98 a/1	0.99 a/1	
	W1		1.65 b/1	2.65 b/1	2.13 a/1	1.79 a/1	2.25 a/1
	W2		1.38 b/1	2.78 b/1	1.48 a/1	2.16 a/1	1.68 a/1
	RT	2.20 a/1	3.25 b/1	2.28 b/1	1.40 a/1	3.15 a/1	
	R1		1.32 b/1	1.54 b/1	2.32 a/1	2.53 a/1	2.63 a/1
	R2		1.42 b/1	2.16 b/1	1.82 a/1	2.87 a/1	2.59 a/1

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$)

(Table 2) was histidine (118.71 in white muscle and 62.90 mg/100 g in red muscle). Decarboxylation of histidine produces histamine, and as in the case of the former, histamine levels were higher in the white muscle. In the case of other biogenic amine-producing FAAs, there were no significant differences of concentration between white and red muscle; in the case of lysine, levels were higher in red than in white muscle. Other authors have reported high histidine concentrations in scombrids in general and in tuna in particular (Wei et al., 1990; Wendakon et al., 1990; Ochiai et al., 1990; Watanabe et al., 1992).

In the course of storage, histidine concentrations decreased significantly ($p < 0.05$) in white muscle but remained stable in red muscle (Table 2). The stability of

Table 3. Essential free amino acids (mg/100 g) in red (R) and white (W) muscle of tuna stored in controlled atmosphere with the gas mixture (1) (60/15/25, $CO_2/O_2/N_2$%) and (2) (40/40/20, $CO_2/O_2/N_2$%) during of the storage until spoilage. T, control lot, stored in air throughout the experiment

Free amino acids	Lots	Days of storage					
		0	5	11	18	25	33
Threonine	WT	3.10 a/1	8.41 a/2	6.15 a/3	8.07 a/2	5.25 a/2	
	W1		4.88 b/1	13.10 b/2	11.89 b/2	17.06 b/3	11.23 a/2
	W2		5.07 b/1	11.93 c/2	4.10 c/1	8.10 c/3	4.51 b/1
	RT	4.43 a/1	9.45 a/2	4.15 c/1	4.90 c/1	7.40 c/3	
	R1		2.50 c/2	2.50 c/2	6.50 c/3	7.25 c/3	12.31 a/4
	R2		2.55 c/2	7.56 c/3	5.30 c/1	7.15 c/3	6.43 b/3
Tryptophan	WT	0.87 a/1	1.54 a/1	0.31 a/1	1.10 a/1	1.20 a/1	
	W1		2.14 a/2	0.40 a/1	1.34 a/1	2.56 b/2	2.88 a/2
	W2		1.15 a/1	0.60 a/1	1.02 a/1	1.75 a/1	1.75 b/1
	RT	0.10 a/1	0.10 b/1	1.85 b/2	0.75 a/1	1.54 a/2	
	R1		0.05 b/1	0.30 a/1	0.25 b/1	1.42 a/2	2.10 a/2
	R2		0.10 b/1	0.64 a/1	1.16 a/2	1.59 a/2	1.25 b/2
Valine	WT	6.07 a/1	14.17 a/2	7.88 a/1	9.07 a/2	7.98 a/2	
	W1		8.65 b/1	20.59 b/2	17.15 b/3	18.69 b/3	15.97 a/4
	W2		7.79 b/1	20.49 b/2	5.48 c/1	11.87 c/3	5.97 b/1
	RT	6.98 a/1	7.68 b/1	4.10 c/2	5.89 c/2	7.72 a/1	
	R1		3.16 c/2	4.65 c/2	5.67 c/2	7.10 a/1	12.65 a/3
	R2		3.98 c/2	8.97 c/3	4.85 c/2	6.85 a/1	6.78 b/1
Methionine	WT	1.68 a/1	4.78 a/2	3.22 a/2	3.75 a/1	4.78 a/1	
	W1		2.93 b/2	7.69 b/3	6.48 b/3	15.13 b/4	8.95 a/3
	W2		3.31 b/2	6.34 b/3	3.49 a/2	6.02 c/3	4.12 b/2
	RT	1.85 a/1	2.60 b/1	1.40 c/1	3.18 a/2	5.10 c/3	
	R1		0.91 c/1	1.61 c/1	2.35 a/2	4.98 c/3	8.98 a/4
	R2		1.68 c/1	3.65 a/2	3.10 a/2	4.15 c/2	5.10 b/3
Isoleucine	WT	2.84 a/1	8.34 a/2	4.25 a/3	4.68 a/3	5.68 a/3	
	W1		5.11 b/2	12.35 b/3	10.69 b/3	17.00 b/4	11.69 a/3
	W2		4.07 b/2	13.07 b/3	3.46 a/2	8.60 c/4	4.17 b/2
	RT	3.97 a/1	4.75 b/1	2.40 a/1	4.50 a/1	7.90 a/2	
	R1		2.19 c/1	2.75 a/1	3.60 a/1	5.71 a/2	11.40 a/3
	R2		2.45 c/1	2.68 a/1	4.10 a/1	6.70 a/2	6.40 b/2
Leucine	WT	5.67 a/1	16.78 a/2	7.08 a/3	8.97 a/3	9.83 a/3	
	W1		7.93 b/1	22.69 b/2	18.12 b/3	31.26 b/4	21.21 a/2
	W2		6.79 b/1	22.19 b/2	6.11 a/1	12.98 c/3	7.61 b/2
	RT	7.70 a/1	8.90 b/1	4.20 c/2	8.09 a/1	14.68 c/3	
	R1		3.40 c/2	5.01 c/2	7.12 a/1	11.50 c/3	22.35 a/4
	R2		4.31 c/2	11.10 d/3	7.40 a/1	12.05 c/3	12.86 c/3
Phenylalanine	WT	1.65 a/1	3.16 a/2	2.79 a/1	5.13 a/3	6.84 a/3	
	W1		3.79 a/2	5.13 b/3	5.91 a/3	15.79 b/4	10.97 a/5
	W2		3.98 a/2	5.26 b/3	4.28 a/3	7.16 a/4	4.69 b/3
	RT	2.80 a/1	2.65 a/1	1.90 a/1	4.90 a/2	8.10 a/3	
	R1		1.58 a/1	2.16 a/1	2.90 a/1	5.98 a/2	11.64 a/3
	R2		2.43 a/1	3.65 a/2	4.35 a/2	6.65 a/3	6.90 b/3

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$)

histidine in red muscle did not correlate with the evolution of histamine in that muscle (Table 1). The other free amino acids (tyrosine, arginine, lysine and ornithine) presented a similar saw-tooth pattern, which was quite different from the pattern of the biogenic amines whose precursors they are. Other authors have likewise found no correlation between these FAAs and biogenic amines (Ababouch et al., 1990; Ruiz-Capillas and Moral, 2002).

Other than in the case of histidine, FAA levels were lowest in the control, followed by the lot stored in an atmosphere with gas mix 2 (Table 2). Decreases in FAA concentrations may be associated with greater consumption of these acids by the microorganisms that use them as a growth substrate, and hence with greater spoilage (Huss, 1995). These results therefore seem to show that, as in the case of biogenic amines, gas mix 1 is much more effective than gas mix 2 as a means of prolonging the shelf life of these tuna species.

Essential free amino acids

The most abundant essential amino acids in the tuna were valine and leucine, with concentrations in excess of 6 mg/100 g (Table 3). Only in the case of tryptophan were

Table 4. Non essential free amino acids (mg/100 g) in red (R) and white (W) muscle of tuna stored in controlled atmosphere with the gas mixture (1) (60/15/25, $CO_2/O_2/N_2$%) and (2) (40/40/20, $CO_2/O_2/N_2$%) during of the storage until spoilage. T, control lot, stored in air throughout the experiment

Free amino acids	Lots	Days of storage					
		0	5	11	18	25	33
Glutamic acid	WT	3.20 a/1	22.06 a/2	22.40 a/2	14.35 a/3	10.65 a/1	
	W1		6.97 b/2	29.68 b/3	28.75 b/3	33.20 b/4	28.45 a/3
	W2		6.37 b/2	20.79 a/3	9.69 c/4	17.65 c/5	8.07 b/4
	RT	16.18 b/1	16.07 c/1	10.15 c/2	12.30 d/2	13.45 a/2	
	R1		8.10 b/2	9.60 c/2	11.97 d/3	12.15 a/3	21.15 c/4
	R2		7.35 b/2	17.21 d/1	8.80 c/2	12.01 a/3	11.90 b/3
Glycine	WT	5.34 a/1	2.25 a/2	7.98 a/3	5.97 a/1	7.54 a/3	
	W1		9.18 b/2	6.15 a/1	5.74 a/1	14.13 b/3	9.11 a/2
	W2		6.00 c/1	4.79 a/1	6.82 a/1	11.09 c/2	4.53 b/1
	RT	15.15 b/1	5.25 c/2	7.82 a/3	4.50 a/2	7.60 a/3	
	R1		7.30 c/2	5.76 a/3	5.34 a/3	6.58 a/2	10.90 a/4
	R2		5.82 c/2	6.32 a/2	6.02 a/2	7.68 a/2	6.60 b/2
Alanine	WT	13.12 a/1	21.15 a/2	17.85 a/3	19.86 a/3	17.83 a/3	
	W1		16.75 b/2	18.64 a/2	17.45 a/2	37.68 b/3	35.04 a/3
	W2		19.04 a/2	25.46 b/3	14.76 b/1	27.01 c/3	13.14 b/1
	RT	41.56 b/1	20.51 a/2	18.15 a/3	15.76 b/3	22.38 d/2	
	R1		17.09 b/2	18.03 a/2	14.97 b/3	17.75 a/2	27.29 c/4
	R2		18.01 b/2	21.25 b/3	15.06 b/4	19.49 a/2	17.89 d/2
β-Alanine	WT	1.42 a/1	1.10 a/1	1.12 a/1	1.78 a/1	1.92 a/1	
	W1		1.12 a/1	1.43 a/1	1.12 a/1	1.34 a/1	0.91 a/1
	W2		2.34 a/1	2.04 a/1	1.58 a/1	1.66 a/1	0.86 a/1
	RT	0.89 b/1	0.69 a/1	0.65 a/1	0.81 a/1	0.90 a/1	
	R1		0.70 a/1	0.89 a/1	0.76 a/1	0.79 a/1	0.78 a/1
	R2		0.98 a/1	0.88 a/1	0.98 a/1	0.78 a/1	0.77 a/1
1-methyl-histidine	WT	3.08 a/1	2.15 a/1	2.14 a/1	3.30 a/1	3.64 a/1	
	W1		3.67 a/1	3.79 a/1	1.97 a/1	2.59 a/1	1.62 a/1
	W2		5.34 a/1	3.55 a/1	2.76 a/1	2.64 a/1	1.98 a/1
	RT	1.35 b/1	1.25 a/1	0.90 a/1	1.90 a/1	1.70 a/1	
	R1		0.92 a/1	1.20 a/1	1.26 a/1	1.53 a/1	1.70 a/1
	R2		1.54 a/1	1.25 a/1	1.78 a/1	1.39 a/1	1.40 a/1
Anserine	WT	228.14 a/1	356.14 a/2	224.19 a/1	265.21 a/3	210.12 a/1	
	W1		140.12 b/2	208.12 b/3	110.09 b/3	164.12 b/4	114.30 a/3
	W2		276.98 c/2	381.45 c/3	121.24 c/4	228.10 c/1	154.58 b/5
	RT	113.78 a/1	107.10 d/2	92.15 d/3	101.25 d/2	118.21 a/1	
	R1		102.16 e/2	122.84 e/3	106.12 d/2	101.65 d/2	108.45 c/2
	R2		111.82 d/2	130.10 f/3	117.21 b/1	113.25 a/1	113.96 a/1

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$)

there significant differences ($p < 0.05$) between concentrations of FAAs in red and white muscle (Table 3). The pattern of evolution of essential FAAs in the course of storage was saw-toothed, like that of the FAAs described above, and agrees with other findings in tuna, hake and Norway lobster (Murata and Sakaguchi, 1986; Ochiai et al., 1990; Watanabe et al., 1992; Yamanaka and Shimada, 1996; Ruiz-Capillas and Moral, 2001b, 2002). Tryptophan has been suggested as a quality index for hake given its increase in ice- and atmosphere-stored hake (Ruiz-Capillas and Moral, 2001b). In the present case, however, there was no such increase, as noted, and hence it will not serve as a quality index for tuna.

Over the storage period, and particularly at the end of that period, essential FAA levels – indicating better quality and hence less spoilage – tended to be higher ($p < 0.05$) in the atmosphere lot stored in gas mix 1. The pattern is the same as for the free amino acids that are converted to biogenic amines by decarboxylation.

Non-essential free amino acids

Levels of non-essential FAAs in red and white muscle of tuna during storage are shown in Table 4. Of these, alanine presented the highest initial levels. Unlike the essential amino acids, concentrations of these FAAs did differ significantly from red to white muscle. Glutamic acid, glycine and alanine concentrations were higher ($p < 0.05$) in red than in white muscle, while the highest concentrations ($p < 0.05$) of β-alanine, 1-methyl histidine and anserine were found in white muscle (Table 4).

Numerous authors have associated glycine, glutamic acid and alanine with characteristic fish flavours (Hayshi et al., 1981; Konosu and Yamaguchi, 1982; Yamanaka and Shimada, 1996). It is therefore reasonable to associate the higher levels of these amino acids with the fact that red muscle has a stronger flavour than white.

As in the case of the other amino acids, the evolution of non-essential FAAs followed a saw-tooth pattern. Again as in the case of essential amino acids, the concentrations of non-essential FAAs were lower in the control and the gas mix 2 lot than in the gas mix 1 lot. As noted earlier, this may reflect the fact that tuna stored in ice or in the mix with the lower CO_2 concentration spoils faster, due to greater microbial growth and hence greater consumption of non-essential FAAs by those microorganisms that use them as a substrate for growth (Ruiz-Capillas and Moral, 2001b).

Concentrations of the dipeptide anserine were very high: 228.14 in white muscle and 113.78 mg/100 g in red muscle. Concentrations of β-alanine and 1-methyl histidine, the components of anserine, were again higher in white than in red muscle (Table 4). Such high levels are associated with osmosis of this compound in the live animal (Abe and Okuma, 1991). Other author also observed longer levels of anserine in tuna (Koriyama et al., 2000). The pattern of anserine evolution during storage was saw-toothed like that of the other amino acids. Concentrations of 1-methyl histidine and β-alanine did not alter significantly in the course of storage (Table 4).

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