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The formation of non-volatile amines in relation to concentrations of free basic amino acids during postmortem storage of the muscle of scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*)

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The concentrations of the non-volatile biogenic amines that form during storage of the adductor muscle of scallop (*Pecten maximus*) and the skeletal muscles of mackerel (*Scomber scombrus*) and herring (*Clupea harengus*) were determined by high-performance liquid chromatography, using postcolumn derivatisation with *o*-phthalaldehyde. Initially, concentrations of amines were low in all muscles but, as time of storage progressed, the concentration of agmatine increased steadily in scallop muscle reaching a level of 100 mg per 100 g wet weight after 10 days of storage in ice or 3 days at 10°C. In mackerel and herring only slight increases were observed, with levels of less than 1.0 mg after 10 days in ice or 2 days at 10°C. In scallop, arginine, the precursor of agmatine, decreased steadily in concentration. In herring muscle, histidine, the precursor of histamine, decreased considerably in concentration, whereas in mackerel it changed only slightly. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Non-volatile amines such as histamine, putrescine, cadaverine, tyramine, tryptamine, spermidine and spermine, the biogenic amines (Guggenheim, 1951), are widely distributed in biological materials, often as regulators of cellular metabolism. For example, the diamine putrescine, and the polyamines spermidine and spermine, are involved in the regulation of nucleic acid function and of protein synthesis. Some of these amines can have important physiological effects; tyramine and phenylethylamine, pressor amines, can cause a rise in blood pressure, whereas histamine, a vasodilator, has the opposite effect (Smith, 1980).

Information available on the presence of these amines in live muscle and muscle of freshly killed fish indicates that their concentrations are very low, if detectable at all, and that it is largely as a result of the action of decarboxylases of spoilage bacteria that concentrations which could be of concern to human health are obtained (Smith, 1980; Taylor, 1986; Clifford & Walker, 1992). Spermine and spermidine are usually the major amines present in fresh muscle and at concentrations of less than 1 mg per 100 g flesh, but, depending upon the species of fish, the free amino acids present in the tissue and the conditions of exposure to spoilage bacteria, other amines such as histamine in fish of the mackerel and herring families (*Scombridae* and *Clupeidae*) can rise to levels in excess of 200 mg per 100 g flesh. (Clifford & Walker, 1992). It is believed that, in these species, free histidine is the precursor of histamine. In molluscs, the amino acid arginine is abundant in the free state and is converted to agmatine by bacterial enzymes during storage (Yamanaka *et al.*, 1987). Similarly, tryptamine can be formed from tryptophan and tyramine from tyrosine. Amines such as putrescine and cadaverine, being low molecular weight compounds, are likely to arise via several biological pathways.

The relationship between the production of these amines and spoilage of fish has been studied extensively (Ritchie & Mackie, 1980; Yamanaka *et al.*, 1987, 1989; Yamanaka, 1989). As the amines are produced by spoilage bacteria, which only appear in significant numbers

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towards the end of the shelf-life of a fish, their concentrations are more likely to be of value as indices of spoilage rather than of freshness. In the EU, for example, a limit of 10 mg per 100 g flesh has been set for the histamine content of fish of the *Scombridae* and *Clupeidae* families at the point of first sale. Other amines such as cadaverine in salmon have been proposed as spoilage indices. However, in squid (*Todarodes pacifius*), agmatine was detected at an early stage of postmortem storage muscle and is of potential value as a 'freshness' index.

High concentrations of amines in foods can be of concern to consumers who have dietary restrictions. Histamine, for example, has been implicated in scombroid poisoning (Taylor, 1986) and, although it is generally the case that the implicated fish have high concentrations of histamine, the aetiology of this form of poisoning remains uncertain (Taylor, 1986; Clifford & Walker, 1992).

This paper is concerned with the formation of biogenic amines in the Atlantic scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) in relation to free basic amino acids in the tissues. It complements previous studies on the formation of agmatine in the adductor muscle of Pacific scallop (*Patinopecten yessoensis*) (Yamanaka, 1989) and the formation of diamines and polyamines during storage of mackerel and herring (Ritchie & Mackie, 1980).

MATERIALS AND METHODS

Scallops (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) were obtained from the Aberdeen Fish Market. The scallops were obtained live; the herring and mackerel were the freshest available and were of A grade quality (Council Regulation, EEC, 1976). On receipt at the laboratory, the adductor muscle was removed from the scallops and the guts were removed from the fish. Three adductor muscles of the scallops and three fish of each species were stored in ice and in a chilled room at 10°C, respectively, and, at intervals, approximately 3.0 g samples were excised for analysis.

Preparation of perchloric acid extracts

Portions of the muscle tissue (approximately 3.0 g) were homogenised in 15 ml of 0.5 M perchloric acid using an Ultra-Turrax T25 homogeniser. The homogenate was filtered through Whatman 2V filter paper and the filtrate (approx. 5.0 ml) passed through 0.2 μm filters to remove any residual particulate material.

HPLC analysis of biogenic amines

The analysis was carried out by a modification of the procedure described by Seiler & Knödgen (1985) using a Varian Vista 5500 high-performance liquid chromatography (HPLC) system coupled to a Varian 9090 Autosampler. The autosampler was fitted with a 20 μl injection loop and the column used was a 150 mm 4.6 mm, 5 μm ODS (C18) Hypersil commercial column (Capital HPLC). The eluant from the column was fed into a T-junction and mixed in a 1:1 ratio with the *o*-phthalaldehyde (OPA)–2-mercaptoethanol reagent. Fluorescence was measured using a Jasco 820 PP fluorimeter with excitation at 300 nm and emission at 950 nm. The electronic signal was fed into a Varian 4270 integrator coupled to a Spectra-Physics Winner (Labnet) computing system.

OPA–2-mercaptoethanol reagent

Boric acid (1.0 M) was titrated to pH 10 with a concentrated solution of potassium hydroxide. To 1 litre of this solution was added 3 ml of Brij, 3 ml of 2-mercaptoethanol and a solution of OPA (1.0 g) in 10 ml of methanol. The reagent was stored in a dark bottle and used within 2 days.

Mobile phase

The mobile phase was as follows:

- Solvent A: 0.1 M sodium acetate, pH 4.5, 10 mM 1-octanesulphonic acid.
- Solvent B: 0.2 M sodium acetate (pH 6.5)–acetonitrile (10:3) with 10 mM octanesulphonic acid.
- Solvent C: methanol.

The gradient elution programme was from 75% solvent A/25% solvent B to 0% solvent A/90% solvent B/10% solvent C at a flow rate of 1.0 ml min^{-1} over a period of 30 min. An appropriate amount of internal standard (1,6-diaminohexane) was added on dilution of the sample as required, and concentrations of the amines were determined by reference to standard solutions. The amounts of standards injected were in the range 25–250 ng for each amine.

Amino acid analysis

An aliquot of 500 μl of each fish extract was added to 200 μl of internal standard (norleucine, ≡20 nmol/ml). Samples were dried in a Speed Vac and then reconstituted in 200 μl of loading buffer. The resultant solution was analysed for free amino acids using an Alpha Plus amino acid analyser.

RESULTS AND DISCUSSION

The concentrations of the amines present in the adductor muscle of the scallop during storage in ice and at 10°C are given in Tables 1 and 2, respectively. They show low initial concentrations of all of the bases in the

Table 1. Concentrations of biogenic amines in the adductor muscle of scallop during storage in ice (mean values, mg per 100 g wet weight)

Storage time (days)	Tym	Put	Cad	Him	Agm	Spd	Spn
0	0	0.35	0.82	0	0.16	0.04	0.54
1	0	0.16	0.31	0	0	0	0.63
3	0	0.07	0.40	0	0.54	0	0.52
6	0	0.24	4.24	0.04	46.7	0	0.56
8	0.67	3.20	9.03	0	88.2	0	0.56
10	1.16	11.4	11.5	0	100	0	0.54

Tym, tyramine; Put, putrescine; Cad, cadaverine; Him, histamine; Agm, agmatine; Spd, spermidine; Spn, spermine.

Table 2. Concentrations of biogenic amines in the adductor muscle of scallop during storage at 10°C (mean values, mg per 100 g wet weight)

Storage time (days)	Tym	Put	Cad	Him	Agm	Spd	Spn
0	0.08	0.35	0.82	0	0.16	0.04	0.54
1	0	0.12	0.85	0	0.43	0	0.49
2	0	0.82	6.14	0	42.2	0	0.46
3	0.39	9.99	9.54	0	112	0	0.45
6	1.47	49.3	19.1	0.23	56.6	0	0.55

Abbreviations as in Table 1.

fresh muscle. As storage time progressed, agmatine became the dominant amine reaching, at 8 days of storage in ice, close to the limit of acceptability for human consumption, a concentration of 88.2 mg per 100 g, and at 10 days 100 mg per 100 g. The other amines, apart from putrescine and cadaverine, changed only slightly, the latter two amines increasing significantly in concentration at the end of the storage life as spoilage bacteria entered the logarithimic phase of growth.

During storage at 10°C, these changes were accelerated showing, after 2 days, similar concentrations of the amines to those obtained after 6 days of storage at 0°C. These findings are generally in agreement with the values obtained by Yamanaka (1989) for concentrations of biogenic amines during storage of the adductor muscle of the Pacific scallop (*Patinopecten yessoensis*) under similar conditions.

Similarly, the results obtained for mackerel and herring (Tables 3 and 4) are in line with earlier observations by Ritchie & Mackie (1980) on the formation of biogenic amines during postmortem storage. It is noteworthy that the levels of histamine reached in mackerel and herring at the end of the storage periods are well below the limit of 10 mg per 100 g set by the EU for those fish at point of first sale. These regulations (Council Directive, EEC, 1991), however, apply to whole ungutted fish, which are known to have faster rates of spoilage.

To enable a direct comparison to be made between the concentrations of the biogenic amines and the basic amino acids in the same samples of fish, selected perchloric acid extracts were analysed for both amino acids and amines. Tables 3–5 give the concentrations of the four basic amino acids arginine, ornithine, lysine and histidine, and the corresponding concentrations of the biogenic amines for the same samples, taken at different periods of storage at 0°C and 10°C. The results for scallop muscle showed arginine to be the dominant basic amino acid. On storage, it decreased by more than 50% at 0°C and by 90% at 10°C (Table 5). At the same time, agmatine increased steadily while putrescine and cadaverine changed only slowly until the latter stages of spoilage. It is assumed that arginine is converted mainly to agmatine, but ornithine is also likely to be a product. Lysine and histidine both increased steadily, presumably due to bacterial breakdown of proteins.

When the same comparison is made with the amino acids and amines in the mackerel and herring extracts (Tables 3 and 4), it is evident that histidine is the dominant free amino acid in both species, but is three times higher in mackerel than in herring. On storage, all of the concentrations of the amino acids decreased with time in herring but, in mackerel, although the trend was the

Table 3. Mackerel muscle: concentrations of basic amino acids and corresponding concentrations of biogenic amines in the same extracts (mg per 100 g wet weight)

Storage time (days)	Fish no.	Amino acids				Biogenic amines						
		Arg	Orn	Lys	Hist	Tym	Put	Cad	Him	Agm	Spd	Spn
Storage in ice												
1	5	3.3	0	28.2	386.2	0	0.06	0	0	0	0.2	0
11	3	18.2	0	17.6	321.7	0.08	0.13	0.24	0.06	0.27	0.25	0
						(0.03)	(0.11)	(0.20)	(0.06)	(0.19)	(0.32)	(0)
Storage at 10°C												
1	3	7.4	0	27.3	373	0	0.12	0.23	0	0.37	0.24	0
2	2	3.6	0	13.9	496	0.58	0.68	2.0	0.85	0.23	0.16	0
						(0.19)	(0.25)	(0.78)	(0.28)	(0.07)	(0.19)	(0)

In parentheses, mean values for three fish stored for the same time.
Abbreviations as in Table 1.

Table 4. Herring muscle: concentrations of basic amino acids and corresponding concentrations of biogenic amines in the same extracts (mg per 100 g wet weight)

Storage time (days)	Fish no.	Amino acids				Biogenic amines						
		Arg	Orn	Lys	Hist	Tym	Put	Cad	Him	Agm	Spd	Spn
Storage in ice												
2	1	3.3	0	15.0	123	0	0.05	0	0	0	0.09	0
4	3	6.0	0	48.5	295	0	0.17	0.23	0	0.38	0.20	0
8	1	3.4	0	25.6	58.2	0	0.21	0.6	0.07	0.61	0.18	0
11	2	2.1	0	24.3	56.7	0.18	0.18	1.71	1.24	0.4	0.06	0
						(0.06)	(0.13)	(0.56)	(0.43)	(0.24)	(0.08)	(0)
13	2	1.5	0	9.2	27.0	0	0.28	4.6	4.5	0.3	0.1	0
						(0.07)	(0.39)	(2.25)	(2.63)	(0.21)	(0.1)	(0)
Storage at 10°C												
1	3	7.5	0	49.9	176	0.09	0.45	3.34	0.71	1.31	0.25	0
2	1	2.4	0	20.9	94.2	1.7	1.0	14.7	23.6	2.0	0.2	0
						(1.17)	(0.83)	(13.6)	(18.6)	(2.42)	(0.17)	(0)

In parentheses, mean value for the three fish stored for the same time.
Abbreviations as in Table 1.

Table 5. Scallop adductor muscle: concentrations of basic amino acids and corresponding concentrations of biogenic amines in the same extracts (mg per 100 g wet weight)

Storage time (days)	Fish no.	Amino acids				Biogenic amines						
		Arg	Orn	Lys	Hist	Tym	Put	Cad	Him	Agm	Spd	Spn
Storage in ice												
3	2	440	7.8	29.8	5.4	0	0.1	0.5	0	0.8	0	0.5
6	3	607	12.9	29.2	7.3	0	0.2	7.8	0.1	93.1	0	0.7
8	3	167	4.4	122	18.0	1.6	5.8	14.9	0	131	0	0.5
10	2	158	2.5	178	25.6	0.7	5.4	9.2	0	116	0	0.5
Storage at 10°C												
1	6	358	0	37.4	10.9	0	0.1	1.5	0	0.4	0	0.3
3	6	125	0	31.4	24.9	0	3.3	8.5	0	153	0	0.4
6	6	39.9	29.8	126	21.0	1.8	47.8	20.2	0.5	102	0	0.4

Abbreviations as in Table 1.

same as for the other amino acids, the values for histidine changed only slightly. The biogenic amine contents reflect those observations, with histamine, in particular, remaining at less than 1.0 mg per 100 g during storage at 0°C and 10°C. In general, amino acid concentrations showed a decrease during postmortem storage. In mackerel and herring, amino acid and protein catabolism must be mainly to nitrogenous substances other than the biogenic amines. In scallop muscle, the amino acids lysine and histidine and the amine agmatine show steady increases in concentrations from an early stage of the storage period. The presence of relatively high concentrations of putrescine and cadaverine, appearing in scallop muscle earlier than in the fish, could indicate early onset of bacterial spoilage.

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