ELSEVIER

PII: S0308-8146(96)00309-3

Food Chemistry, Vol. 60, No. 1, pp. 57-59, 1997

0308-8146/97 $17.00+0.00

Biogenic amine composition of the gonads of herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and scallop (*Pecten maximus*)

I. M. Mackie,* L. Pirie & H. Yamanaka†

Food Science Laboratory, Torry, P.O. Box 31, 135 Abbey Road, Aberdeen, UK

(Received 28 June 1996; revised version received 5 September 1996; accepted 5 September 1996)

The concentrations of the biogenic amines, tyramine, putrescine, cadaverine, histamine, agmatine, spermine and spermidine, present in the gonads of herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and scallop (*Pecten maximus*), were determined in 0.5 M perchloric acid extracts by high-performance liquid chromatography using postcolumn derivatisation with *o*-phthalaldehyde. The highest overall concentrations were in mackerel gonads and the lowest in herring. Agmatine and spermine were each present in mackerel tissues at concentrations greater than 20 mg per 100 g. The total amounts of amines in the testes of scallop were, on average, 9 mg per 100 g greater than in the ovaries; tyramine and agmatine were of higher concentrations in the testes. Histamine was absent in herring gonads and, if present in those of other species, was at concentrations of less than 1.0 mg per 100 g. © 1997 Elsevier Science Ltd

INTRODUCTION

Biogenic amines are found widely in biological materials of animals, plants and microorganisms, often associated with the control of cellular metabolism (Guggenheim, 1951). They are generally low molecular weight compounds such as the simple aliphatic primary amines, putrescine and cadaverine, or aromatic amines such as phenylethylamine or tyramine (Smith, 1980). Because of their strong basicity, they can act to neutralise the strong acidity of DNA in cells and are often identified with the male and female generative cells.

Because amines, in general, can be produced as a result of the action of bacterial decarboxylases, it is frequently observed that, on spoilage of fish or maturation of wines and cheeses, relatively high concentrations can be produced (Smith, 1980; Taylor, 1986). In fish, for example, histamine is produced by bacterial decarboxylation of free histidine present in mackerel and other pelagic species (Fernandez-Salguero & Mackie, 1979). However, although often associated with scombroid poisoning (Ahmed, 1991), it has not been shown to be directly responsible (Clifford & Walker, 1992). Another amine, tyramine, often found in cheese, is formed by decarboxylation of tyrosine. As these amines can have pronounced physiological effects on humans—tyramine causes an increase in blood pressure, whereas histamine is a vasodilator—it is important that the consumer is aware of their presence in food and that, should there be dietary restrictions, he or she can avoid eating them (Lessof, 1995).

In this paper, the concentrations of biogenic amines in the gonads of scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) are reported.

New address: Rowett Research Institute, Greenburn Rd, Bucksburn, Aberdeen AB21 9SB.
Visiting Scientist from The Department of Food Science and Technology, Tokyo University of Fisheries, Tokyo, Japan.

MATERIALS AND METHODS

Fish and shellfish samples

The herring, mackerel and scallops were all obtained from the Aberdeen Fish Market. The herring and mackerel were the freshest available and were of A grade quality (EEC, 1976). The scallops were obtained live. On receipt at the laboratory, the gonads were removed, weighed and then kept chilled in ice prior to extraction for analysis.

Preparation of perchloric acid extracts

Portions of the gonads (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) was 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 Auto Sampler. 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 octane sulphonic 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.

Table 1. Herring: concentrations of biogenic amines in gonads (mg free base per 100 g wet weight)

Sample	Tym	Put	Cad	Him	Agm	Spd	Spn	Total
86, T	0	2.58	0	0	0.66	2.96	5.96	12.2
87, O	0	2.74	0.40	0	0.73	3.03	1.21	8.11
88, O	0	6.04	0.20	0	0.45	4.13	2.58	13.4
89, O	0	5.41	0.86	0	2.29	4.00	2.14	14.7
90, T	0	3.07	0.14	0	0.88	3.62	6.04	13.8
91, T	0	3.33	0	0	0	4.82	4.91	13.1

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

RESULTS AND DISCUSSION

The amine concentrations obtained are likely to be as close to values present in the living tissues as it is possible to achieve using commercial sources of fish and shellfish. This applies particularly to the values obtained for scallop gonads as the extractions were done immediately after removal from the live animals. For the fish, some postmortem spoilage changes will have taken place, but these must be only to a small extent as the fish selected were in very fresh condition. As histamine formation from free histidine is often an indicator of bacterial spoilage (Fernandez-Salguero & Mackie, 1979; Yamanaka, 1989), its absence or presence only in low concentrations in all of the samples is consistent with the postmortem histories.

When the total compositions of the amines in the gonads of the two fish species are compared (Table 1–3), those for mackerel (Table 2) are considerably greater than for herring (Table 1), the difference being accounted for mostly by the high concentrations of agmatine, spermine and cadaverine in mackerel.

Table 2. Mackerel: concentrations of biogenic amines in gonads (mg free base per 100 g wet weight)

Sample	Tym	Put	Cad	Him	Agm	Spd	Spn	Total
92, O	2.63	4.68	11.1	0.26	19.4	4.67	22.1	64.8
93, O	1.94	2.37	7.72	0	24.00	3.52	26.9	66.42

Abbreviations as in Table 1.

Table 3. Scallop: concentrations of biogenic amines in gonads (mg free base per 100 g wet weight)

Sample	Tym	Put	Cad	Him	Agm	Spd	Spn	Total
94, O	0.27	3.04	4.00	0	0.43	0.62	3.66	12.0
U								
95, T	2.79	4.60	12.7	0	2.99	0.71	7.96	13.7
96, O	0	5.79	5.95	0	0	0.94	9.08	21.8
V								
97, T	1.00	4.40	16.4	0	2.80	0.54	5.55	30.7
98, O	0.26	3.56	4.56	0.14	0.34	0.89	4.50	14.3
W								
99, T	1.32	3.37	6.58	0.43	1.80	0.27	8.70	22.5
100, O	2.78	9.24	9.43	0.90	0.63	1.03	8.59	32.6
X								
101, T	4.10	6.31	10.7	0.92	1.13	0.36	6.92	30.9
102, O	0.78	2.76	6.61	0.17	2.03	0.82	5.07	18.2
Y								
103, T	4.98	3.44	13.2	0.50	5.71	0.31	8.70	36.9
104, O	0	5.22	6.40	0	0	1.06	8.74	21.4
Z								
105, T	0.83	3.68	5.17	0	0	0.43	12.55	22.7

Abbreviations as in Table 1.

From the data available, it is also evident that there are no sex-related differences in the total amounts of bases present in the fish. For herring, for which a comparison is possible, the specific amine composition does not differ between roe or milt. However, the results for the organs of the scallop (Table 3), which is an hermaphrodite, show that the total amounts of amines in the testes are on average 9–100 g greater than in the ovaries. There was no relationship with the level of maturity of the organs, viz. samples U and Z (immature) compared with samples W and X (mature). With regard to specific amine differences between the gonads of scallop, tyramine and agmatine were of higher concentration in the testes than in the ovaries.

Overall, the biogenic amine contents of the gonads examined were of low concentration, with the notable exception of agmatine and spermine in mackerel tissue with levels greater than 20 mg per 100 g. During post-mortem storage of squid, agmatine has been shown to be produced from free arginine present in squid (Yamanaka *et al.*, 1987). Cadaverine, although to a lesser extent than the above amines, was also present in relatively high concentrations in the mackerel tissues.

In herring, the contents of the amines were low in contrast to those of mackerel and, if there are sex-related differences, spermine levels are higher in the testes than in the ovaries. However, because of the small number of samples analysed, there are insufficient data from which to draw more definite conclusions.

A paper by Takeda *et al.* (1982) reported concentrations greater than 2 mg per 100 g for spermine and spermidine in cod roe. As far as the authors are aware, there are no other published reports on the biogenic amine contents of the gonads of fish.

REFERENCES

Ahmed, F. E., ed. (1991). *Seafood Safety*. Committee on Evaluation of Safety of Fishery Products, Institute of Medicine, National Academy of Sciences. National Academy Press, Washington, DC.

Clifford, M. N. & Walker, R. (1992). The aetiology of scombrotoxicosis. *Int. J. Food Sci. Technol.*, **27**, 721–724.

EEC (1976). Laying down common marketing standards for certain fresh or chilled fishery products. Council Regulation (EEC) No. 103/76, 19 January (1976).

Guggenheim, M. (1951). *Die Biogenic Amine*. Karger, Basel.

Fernandez-Salguero, J. & Mackie, I. M. (1979). Histidine metabolism in mackerel (*Scomber scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions. *J. Food Technol.*, **14**, 131–139.

Lessof, M. (1995). Adverse reactions to foods. *BNF Nutr. Bull.*, **20**(73), 16–25.

Seiler, N. & Knödgen, B. (1985). Determination of polyamines and related compounds by reversed phase high performance liquid chromatography: improved separation system. *J. Chromatogr.*, **339**, 43–57.

Smith, T. A. (1980). Amines in food. *Food Chem.*, **6**, 169–200.

Takeda, Y., Abe, F. & Samejima, K. (1982). Spermidine and spermine contents in foodstuffs. *J. Hyg. Chem.*, **28**(5), 279–281.

Taylor, S. L. (1986). Histamine food poisoning: toxicology and clinical aspects. *CRC Crit. Rev. Toxicol.*, **17**, 91–128.

Yamanaka, H. (1989). Changes in polyamines and amino acids in scallop adductor muscle during storage. *J. Food Sci.*, **59**(5), 1133–1138.

Yamanaka, H., Shiomi, K. & Kikuchi, T. (1987). Agmatine as a potential index of freshness of common squid (*Todarodes pacificus*). *J. Food Sci.*, **52**, 936–938.