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Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging

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Abstract

A comparative study of the effects of packaging on the formation of biogenic amines during storage of sardines (*Sardina pilchardus*) at 4 °C in air, modified atmosphere pack (MAP) and vacuum pack (VP) was carried out. Sardines were organoleptically acceptable for up to 3 days in air, 12 days in MAP and 9 days in VP. The biogenic amine content generally increased in all treatments with increasing storage time. The concentrations of putrescine and/cadaverine in fish stored in air reached maximum levels of 12.2 mg/100g at 12 days and 10.0 mg/l00 g at 15 days. Significant differences were found ($P < 0.05$) in the levels of cadaverine and putrescine among the three treatments. Spermidine and spermine levels increased slightly and did not change much throughout the storage period for all experimental conditions. The amine contents of sardine were highest in sardine stored in air, followed by VP and MAP. Quality indices related to the contents of the major biogenic amines were calculated and they correlated well with organoleptic qualities.

Keywords: Sardine; Gas pack; Vacuum pack; Biogenic amines

1. Introduction

Modified atmosphere pack (MAP) is a form of packaging involving the removal of air from the pack and the replacement with a single gas or mixture of gases. The gaseous atmosphere changes continuously during storage because of respiration of the packed product, biochemical changes and the slow permeation of gases through the packaging materials (Parry, 1993). Vacuum pack (VP) is also a type of MAP system because air is removed from a pack and not replaced. VP is normally placed in a pack of low oxygen permeability, air is evacuated and the package sealed (Church, 1998). An evacuated VP pack collapses around the product so that the pressure inside is a little less than atmospheric. The gaseous atmosphere of VP is likely to change throughout storage; hence the pack atmosphere is modified indirectly.

The use of a modified atmosphere with carbon dioxide has been shown to extend the shelf-life of foods by inhibiting microbial growth (Farber, 1991). Though MAP technology has a potential seafood safety risk related to *Clostridium botulinum* type E, consumer preferences for usefulness in handling, good visual display, attractive and safe packaging, hygiene and extension of acceptable shelf-life of seafood products have continued to increase. Commercial and regulatory interest in packaging technology is not a new concept but innovative developments in modified atmosphere and vacuum-packing system, such as packaging materials, safety indicators, machinery and related sensor technology, could provide further improvement in seafood shelf-life, organoleptic quality, and product range.

Biogenic amine formation in seafood is important as histamine, and possibly other biogenic amines, are respon-

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sible for scombrotoxic fish poisoning (Taylor, 1986). Furthermore, biogenic amines, have been used as chemical indicators of seafood quality (Jorgensen, Huss, & Dalgaard, 2000; Mieltz & Karmas, 1977, 1978). Fish muscle is capable of supporting the bacterial formation of a wide variety of amine compounds that come from the decarboxylation of amino acids. Biogenic amine formation in fish and fish products depends on the amino acid content of fish, the presence of bacterial biogenic amine decarboxylases and favourable environmental conditions (Brink, Damink, Joosten, & Huis in't Veld, 1990; Shalaby, 1996; Silla-Santos, 1996; Tarjan & Janossy, 1978). Certain types of bacteria produce decarboxylase enzymes, which act on free amino acids in the fish muscles during spoilage.

Fish are different from other MAP products in terms of their biological structure and chemical composition since they are extremely perishable and generally spoil faster than other muscle foods. The appearance of the seafood product in MAP/VP is important because it influences the acceptability of the product to consumers. The appearance of a seafood product can be assessed visually, including pack collapse, production of drip, and discoloration. Organoleptic properties of the product can be assessed by colour, odour, flavour and texture. The effects of MAP on seafood have been reviewed extensively (Davis, 1993; Farber, 1991; Reddy, Armstrong, Rhodehamel, & Kauter, 1992; Sivertsvik, Jeksrund, & Rosnes, 2002; Stammen, Gerdes, & Caporaso, 1990) but little information exists regarding biogenic amine production in sardines stored in modified atmospheres and vacuum packs. Therefore, in the present investigation, a comparative study of the effect of modified atmosphere and VP on biogenic amine formation in sardine (*Sardina pilchardus*), with an emphasis on a potential quality index, was attempted.

2. Materials and methods

2.1. Packaging and storage of sardine

Sardines (*Sardina pilchardus*) obtained from Scotland (UK), were two days post-capture on arrival at the laboratory in ice. They were immediately gutted and divided into three lots. One lot was stored in air and the remaining two lots were placed in nylon-polyethylene pouches (30×35 cm); the second lot was vacuum-packed and the third lot was gas-packed in a Multivac model A 300 vacuum-packaging machine (Bury, Lancs., UK). The O_2 transmission rate of pouches was 47 cm^3/m^2 24 h. The gas ratio was 60% CO_2 and 40% N_2, typical for packing fatty fish in MAP (Cann, Smith, & Houston, 1983). The final gas/sample ratio in all pouches was about 2:1 (v/w) for MAP conditions. All samples were stored in a refrigerator with controlled temperature (4 °C). Six fish were removed from each batch for each sampling but data were obtained using three samples (3×2) for biogenic amines analysis.

2.2. Sample preparation

Fish muscle (5 g) was taken from the dorsal part of the sardines, without skin, and transferred to a 250 ml centrifuge tube. The sample was then extracted with 20 ml of 6%TCA for 3 min, centrifuged at 12,000 rpm for 10 min at 4 °C and filtered through Whatman No. 1 filter paper. An aliquot was made up to 50 ml with distilled water and was stored in a refrigerator until injection on the HPLC.

2.3. Analytical method

Derivatization procedure, chromatographic condition and quantification of biogenic amines were done according to the method of Özogul (2002a, 2002b).

2.4. Apparatus and columns

HPLC analyses were performed with a Merck–Hitachi Model D-6500 (Merck Ltd., Poole, Dorset, UK) apparatus equipped with a diode array detector (Merck–Hitachi L-4500) and an intelligent pump (Merck–Hitachi L-6200A). The column was a Waters Spherisorb ODS-2 C_{18} (125×4.60 mm, particle diameter 5 μm).

2.5. Reagents

All biogenic amine standards and benzoyl chloride were purchased from Sigma–Aldrich (Poole, Dorset, UK). The mobile phase consisted of acetonitrile and HPLC grade water (Philip Harris Scientific, Lichfield, Staffordshire, UK) for biogenic amines determination.

2.6. Quality index (QI)

The quality index and the biogenic amine index were calculated according to the procedures described by Mieltz and Karmas (1977), Veciana-Nogues, Marine-Font, and Vidal-Carou (1997), respectively. The formulas used were as follows:

$$\text{Quality index} = (\text{histamine} + \text{putrescine} + \text{cadaverine}) / (1 + \text{spermidine} + \text{spermine})$$

$$\text{Biogenic amine index (BAI)} = (\text{histamine} + \text{putrescine} + \text{cadaverine} + \text{tyramine})$$

2.7. Sensory evaluation

Sensory evaluation was carried out according to the Branch and Vail (1985) sensory assessment scheme, as modified by Özogul, Taylor, Quantick, and Özogul (2000) for herring. This sensory assessment approach evaluates freshness by giving demerit points according to certain aspects of general appearances (e.g. skin, slime, scales, eyes, gills, belly). Each assessment was carried out

by a minimum of six trained panellists. Panellists were asked to state whether or not the fish were acceptable. This was used to determine the shelf-life of the fish. The acceptable shelf-life was found to correspond to a demerit score of 17 ± 2. Duplicate samples from each of the three storage conditions were taken at regular intervals.

2.8. Statistical analysis

The Student's *t*-test and standard deviation were used for data analysis. Significance of differences was defined as $P \leqslant 0.05$. Statistical comparison was based on three samples for each treatment for each specific storage time.

3. Results and discussion

3.1. Sensory effects

Sensory analysis, that was presented in previous work (Özogul, Polat, & Özogul, 2004) revealed that sardine kept under three different storage conditions were still edible at 3 days in air, 9 days in VP and 12 days in MAP. The initial quality characteristics of the sardine were very fresh, seaweedy odours, firm texture and glossy appearance. The acceptable shelf-life was found to correspond with a demerit score of 17 ± 2. The observed demerit score was 16–17 in air, 17–18 in VP and 19 in MAP. The rate of rise of demerit points was fairly linear with storage time under all three conditions. Significant differences ($P < 0.05$) were found in the levels of demerit points between sardine stored in air and in VP, especially in MAP. The progress of decomposition of sardine showed decomposed off-odour after 3 days in air, 9 days in VP and 12 days in MAP, since sardines have high red meat and fat compositions. Ababouch et al. (1996) found that the keeping time of sardines changed from 21 and 27 h for fish stored at ambient temperature to 9.5 days in ice. Similar results were obtained by El Marrakchi, Bennour, Bouchriti, Hamama and Tagafait (1990) for iced sardine (9 days).

3.2. Biogenic amines analysis

The concentrations of biogenic amines in sardines stored in air, MAP and VP for 15 days at 4 °C are shown in Tables 1–3, respectively. The levels of histamine and TMA under the different storage conditions were given in the first part of this study (Özogul et al., 2004). The amount of histamine increased during the storage period and reached 20.3 ± 1.3 mg/100 g for air storage, 14.0 ± 1.2 mg/100 g for VP and 10.5 ± 1.2 mg/100 g for MAP. The increase of TMA samples in air was higher than in samples stored in VP and MAP throughout the time of storage, reaching the levels of 25.5 mg/100 g for air storage, 19.2 mg/100 g for VP and 14.3 mg/100 g for MAP.

Large changes in the contents of putrescine and cadaverine were observed throughout the storage period of sar-

Table 1
Biogenic amine contents of sardine stored in air (mg/100 g)

Storage time (d)	Putrescine	Cadaverine	Spermidine	Spermine	2-Phenyl-ethylamine	Tryptamine	Tyramine	Agmatine
0	1.34 ± 0.6	0.39 ± 0.2	0.12 ± 0.1	0.00	0.00	ND	0.00	0.00
2	1.96 ± 1.5	0.42 ± 0.3	0.28 ± 0.1	0.08 ± 0.1	0.34 ± 0.1	ND	1.28 ± 0.5	0.14 ± 0.1
4	2.58 ± 1.8	1.46 ± 0.4	0.35 ± 0.2	0.17 ± 0.1	0.45 ± 0.2	ND	2.57 ± 1.5	0.56 ± 0.4
6	4.72 ± 2.6	3.69 ± 2.7	0.49 ± 0.3	0.23 ± 0.2	0.68 ± 0.5	ND	4.63 ± 2.8	0.98 ± 0.7
8	6.83 ± 3.5	4.05 ± 3.8	0.68 ± 0.5	0.31 ± 0.2	0.27 ± 0.2	ND	7.92 ± 3.3	1.34 ± 1.0
10	7.45 ± 4.0	6.13 ± 5.0	0.66 ± 0.4	0.27 ± 0.1	0.53 ± 0.4	ND	5.61 ± 2.7	1.91 ± 1.6
12	12.2 ± 4.6	8.32 ± 4.2	0.72 ± 0.3	0.43 ± 0.2	0.00	ND	2.29 ± 3.4	2.35 ± 17.5
15	11.4 ± 2.6	10.04 ± 4.9	0.76 ± 0.2	0.29 ± 0.2	0.00	ND	1.63 ± 1.7	2.65 ± 2.3

The values are expressed as means ±SD ($n = 3$).
ND, not detected.

Table 2
Biogenic amine contents of sardine stored in MAP (mg/100 g)

Storage time (d)	Putrescine	Cadaverine	Spermidine	Spermine	2-Phenyl-ethylamine	Tryptamine	Tyramine	Agmatine
0	NT	NT	NT	NT	NT	NT	NT	NT
2	0.43 ± 0.3	0.08 ± 0.1	0.04 ± 0.0	0.00	0.23 ± 0.9	ND	0.93 ± 0.6	0.00
4	1.24 ± 0.8	0.35 ± 0.2	0.23 ± 0.1	0.07 ± 0.1	0.00	ND	1.36 ± 1.1	0.00
6	1.94 ± 1.1	1.04 ± 0.9	0.29 ± 0.2	0.15 ± 0.1	0.38 ± 0.2	ND	2.49 ± 2.0	0.43 ± 0.3
8	2.76 ± 1.6	2.15 ± 1.4	0.46 ± 0.3	0.26 ± 0.1	0.27 ± 0.1	ND	1.42 ± 1.2	0.89 ± 0.5
10	5.83 ± 2.5	5.21 ± 4.3	0.58 ± 0.5	0.34 ± 0.2	0.14 ± 0.1	ND	0.58 ± 0.3	1.07 ± 1.1
12	4.68 ± 3.5	4.02 ± 3.5	0.53 ± 0.4	0.29 ± 0.1	0.00	ND	0.00	1.86 ± 1.6
15	2.06 ± 1.4	1.82 ± 1.0	0.47 ± 0.3	0.26 ± 0.1	0.00	ND	0.00	1.45 ± 1.3

The values are expressed as means ±SD ($n = 3$).
NT, not tested.
ND, not detected.

Table 3
Biogenic amine contents of sardine stored in VP (mg/100 g)

Storage time (d)	Putrescine	Cadaverine	Spermidine	Spermine	2-Phenyl-ethylamine	Tryptamine	Tyramine	Agmatine
0	NT	NT	NT	NT	NT	NT	NT	NT
2	0.89 ± 0.4	0.15 ± 0.1	0.12 ± 0.1	0.05 ± 0.0	0.46 ± 0.2	ND	1.04 ± 0.8	0.00
4	1.48 ± 1.1	0.75 ± 0.5	0.28 ± 0.2	0.11 ± 0.1	0.24 ± 0.1	ND	3.59 ± 3.5	0.08 ± 0.0
6	4.63 ± 2.4	2.84 ± 1.9	0.38 ± 0.3	0.18 ± 0.1	0.53 ± 0.4	ND	3.72 ± 2.6	0.29 ± 0.1
8	5.56 ± 3.8	3.58 ± 2.2	0.53 ± 0.4	0.32 ± 0.2	0.18 ± 0.1	ND	4.26 ± 3.3	0.54 ± 0.4
10	7.59 ± 4.1	5.73 ± 4.6	0.61 ± 0.5	0.39 ± 0.2	0.00	ND	3.26 ± 1.8	1.76 ± 1.5
12	5.06 ± 3.6	4.73 ± 3.8	0.57 ± 0.4	0.35 ± 0.3	0.23 ± 0.1	ND	2.59 ± 1.9	2.06 ± 1.7
15	4.67 ± 2.7	3.62 ± 2.7	0.52 ± 0.4	0.31 ± 0.2	0.00	ND	1.45 ± 1.0	2.32 ± 2.0

The values are expressed as means ±SD ($n = 3$).
NT, not tested.
ND, not detected.

dine in MAP, VP and in air storage. The concentration of putresine increased during the storage period of sardine held in air, VP and MAP, reaching maximum levels of 12.2 mg/100 g at 12 days, 7.59 mg/100 g at 10 days and 5.83 mg/100 g at 10 days, respectively, whereas cadaverine levels reached of maximum level of 10.0 mg/100 g for fish stored in air for 15 days, 5.73 mg/100 g in VP for 10 days and 5.21 mg/100 g in MAP for 10 days of storage. Significant differences were found ($P < 0.05$) in the levels of cadaverine and putresine among the three treatments. Ababouch et al. (1996) found that cadaverine and putresine accumulated rapidly, reaching levels of 235 mg/100 g and 30 mg/100 g, respectively, after 24 h of storage at ambient temperature. In this experiment, these amine contents of sardine were highest in sardine stored in air, followed by VP and MAP. These findings were similar to our previous work on herring stored in air and MAP at 2 ± 2 °C. It was found that the concentrations of these amines in herring held at 2 ± 2 °C increased more rapidly than in herring stored in MAP (Özogul, 2002a, 2002b; Özogul et al., 2004).

Spermidine and spermine levels increased slightly and did not change much throughout the storage period for the three different conditions. No significant differences were observed in spermine and spermidine contents among the treatments ($P > 0.05$). However, lower values were obtained for sardine stored in MAP (Table 2). This is in agreement with our previous findings for herring stored in MAP (Özogul, 2002a, 2002b). Ababouch et al. (1996) reported that ice storage inhibited the formation of these two amines in sardine. However, at ambient temperature, spermine and spermidine levels reached 5 and 6 mg/100 g after 24 h, respectively.

2-Phenylethylamine appeared after two days, for all storage conditions, and its concentration increased from 0.34 mg/100 g to 0.68 mg/100 g after six days of air storage. After that, its level decreased to 0.53 mg/100 g and it was not detected at 12 days. Similar trends were obtained for sardine stored in MAP and VP. Tryptamine was not detected in any samples. Tyramine concentration increased during the storage period, reaching the levels of 7.92 mg/100 g for sardine in air at eight days, 4.26 mg/100 g for VP at eight days and 2.49 mg/100 g for MAP at six days. After that, the level of tyramine started to decrease for all storage conditions. Agmatine was not detected for sardine in air and VP until two days but six days for MAP. The levels of agmatine increased throughout the storage period except for MAP, the level of agmatine decreased after 12 days of MAP storage (Table 2). There are no significant differences within the three storage conditions ($P > 0.05$).

Table 4 shows biogenic amine quality and indices for sardine stored in air, VP and MAP. There was an increase in the two indices with storage time, indicating that these

Table 4
Quality and biogenic amine indices of sardine stored in air, MAP and VP

Storage time (d)	AIR		MAP		VP	
	Quality index	Biogenic amine index	Quality index	Biogenic amine index	Quality index	Biogenic amine Index
0	3.11	3.48	NT	NT	NT	NT
2	5.37	8.58	2.45	3.48	3.05	4.61
4	9.26	16.7	3.66	6.12	4.68	10.1
6	11.7	24.8	5.03	9.73	8.39	16.8
8	12.7	33.2	6.26	12.2	9.36	21.6
10	15.7	35.9	9.94	19.7	12.0	27.2
12	18.5	42.0	9.96	18.1	11.1	24.0
15	20.3	43.3	8.32	14.4	12.2	23.7

Quality index = (histamine + putrescine + cadaverine)/ (1 + spermidine + spermine).
Biogenic amine index (BAI) = (histamine + putrescine + cadaverine + tyramine).
NT, not tested.

two indices can be used to determine the spoilage of sardine. However, the biogenic amine index gave values twice higher than the quality index. Sensory analysis, presented in our previous work (Özogul et al., 2004), showed that sardines were edible at 3 days in air, 9 days in VP and 12 days in MAP, at which time quality indices were <9.26, <12.0 and 9.96, respectively, whereas biogenic amine indices were <16.6 for air, <27.2 for VP and 18.1 for MAP storage, respectively (Tables 1–3). Mieltz and Karmas (1977) proposed the value of 10 as the limit of fish acceptability for the quality index (QI). This value was reached in samples after 4 days of storage for air, 12 days for MAP and 8 days for VP, indicating that the quality index correlated well with organoleptic quality of sardines stored in air, MAP and VP. The biogenic amine index (BAI) proposed by Veciana-Nogues et al. (1997) also correlated with sensory acceptability of sardine in air, MAP and VP since it increased with storage time. However, more studies on BAI are needed to set a limit of fish acceptability in MAP and VP.

Consequently, fish in air exhibited higher biogenic amine values than in VP and MAP storage. The use of a modified atmosphere (MAP) with carbon dioxide has been shown to extend the shelf-life (approximately three days) of sardine by inhibiting microbial growth compared to VP. It can also be concluded that the biogenic amine index gave values twice as high as the quality index.

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