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The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*)

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Abstract

Chemical, sensory and microbiological evaluation of sardine (*Sardina pilchardus*), with emphasis on the quality and safety parameters in modified atmosphere packaging (MAP) and vacuum packaging (VP), were investigated. Quality assessment of sardines stored in MAP (60%CO_2:40%N_2) and VP for up to 15 days at 4 °C was done by the monitoring of sensory quality, total viable counts (TVC), nucleotide degradation products, histamine, trimethylamine (TMA) and total volatile base nitrogen (TVB-N). The observed shelf life of sardine was found to be 12 days in MAP, 9 days in VP and 3 days in air. Bacteria grew most quickly in sardine stored in air, followed by those in VP and the lowest counts were with MAP. The concentration of histamine increased and its level reached over 20 mg/100 g for fish stored in air, 13 mg/100 g for VP and 10 mg/100 g for MAP at 15 days. The highest concentration of TMA was obtained from sardine stored in air, followed by sardine stored in VP and the lowest in MAP. The formation of TVB-N increased with time of storage. When the TVC had reached 10^6 cfu/g, the TVB-N content was found to be approximately 15 mg/100 g muscle for all storage conditions.

Keywords: Sardine; Modified atmosphere pack; Vacuum pack; Histamine; TMA; TVB-N and K-value

1. Introduction

Fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms. Modification of the atmosphere within the package by decreasing the oxygen concentration, while increasing the content of carbon dioxide and/or nitrogen, has been shown to significantly prolong the shelf life of perishable food products at chill temperatures (Parry, 1993). Modified atmosphere packaging (MAP) and vacuum-packaging (VP), along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands. MAP and VP systems could provide further improvement in seafood shelf life, organoleptic quality, and product range. However, undoubtedly the single most important concern with the use of modified atmosphere and VP products is the potential for the outgrowth and toxin production by the non-proteolytic, *Clostridium botulinum* type E which can grow at low temperatures. In addition to this, pack collapse, increased exudates/drip loss, discoloration, and histamine production are major potential problems during the storage of fish and shellfish products in MAP (Church, 1998).

The shelf life of fish products in MAP can be extended, depending on raw materials, temperature, gas mixtures and packaging materials (Farber, 1991). The percentage increase of shelf life in MAP ranges from 0 to 280%, compared with aerobic storage (Reddy, Armstrong, Rhodehamel, & Kauter, 1992).

The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour, odour and texture. Inhibition of the growth of these microorganisms and increase in the lag phase of facultative and anaerobic microorganisms results in an increase in the potential shelf life of MAP

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products that is one of the main benefits of MAP technology. Nevertheless, there is a safety concern with extended shelf life because of the growth of pathogenic organisms in these packaging systems (Church & Parsons, 1995; Farber, 1991).

Trimethylamine (TMA) content (Tozawa, Enokihara, & Amano, 1971), total volatile bases (TVB) (Antonacopoulos & Vyncke, 1989), individual nucleotides (Hattula, Kievaara, & Moran, 1993; Jacober & Rand, 1982) and nucleotide ratios (K, Ki, H and G-values) have been proposed as indices of deterioration of fish quality (Burns, Ke, & Irvine, 1985; Ehira & Uchiyama 1987; Karube, Matsuoka, Suzuki, Watanabe, & Toyama, 1984; Luong, Male, Masson, & Nguyen, 1992). Levels of biogenic amines can also be useful in estimating freshness or degree of spoilage of fish since their formation is associated with bacterial spoilage (Mackie, Pirie, Ritchie, & Yamanaka, 1997)

Fish containing relatively high concentrations of histamine can cause poisoning or allergic reactions when consumed by some individuals. Histamine is produced by microbial decarboxylation of the amino acid histidine. The importance of estimating the concentration of histamine in fish and fish products is related to its impact on human health and food quality. Histamine formation in MAP has been found to be lower than in air (Özogul, Taylor, Quantick, & Özogul, 2002b; Watts & Brown, 1982)

The effects of modified atmosphere packaging on seafood have been reviewed extensively (Davis, 1993; Farber, 1991; Reddy et al., 1992) but little information is available on storage of sardines in modified atmosphere packing. Therefore, the main objective of this study was to investigate the effects of modified atmosphere-packaging and vacuum-packaging on the quality and safety of sardines. Quality attributes were assessed by different methods, including chemical, microbiological, and sensory evaluation.

2. Materials and methods

2.1. Packaging and storage of sardine

Sardines (*Sardina pilchardus*) obtained from Mallaig in Scotland, were 2 days post-capture on arrival at the laboratory in ice. They were immediately gutted and divided into three lots in ice. Time of the gutting and division into lots was 3 h. 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 modified atmosphere-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). For the experiment, approximately 15 kg fish were used and, on each occasion, 600–700 g fish was sampled. Six fish were removed from each batch for each sampling but data were obtained using three samples (3×2) for chemical analysis (two fish were minced for each sampling).

2.2. Proximate analysis

Lipid content of fish was determined by the Bligh and Dyer (1959) method. Moisture content of fish was determined by the AOAC (1983) method. Total crude protein was determined by the semi-micro Kjeldahl method (Egan, Kirk, & Sawyer, 1981). The ash content was determined by charring over a Bunsen burner and then placing in a furnace at 550 °C (for 12 h) to incinerate until the sample was free of carbon particles.

2.3. Analytical method

The TVB content was measured according to the method of Antonacopoulos and Vyncke (1989) and expressed as mg TVB-N per 100 g fish muscle. TMA and histamine were determined using the method of Özogul, Taylor, Quantick, and Özogul (2002a). ATP and its breakdown products were determined according to the method of Özogul et al. (2000a).

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). For histamine determination, the column was a Waters Spherisorb ODS-2 C_{18} (125×4.60 mm, particle diameter 5 μm). For determination of nucleotides, the column was a Sphereclone ODS 2 C_{18}, 150×4.60 mm, particle diameter 5 μm. Both columns were purchased from Phenomenex (Macclesfield, Cheshire, UK).

2.5. Reagents

Histamine, TMA, all nucleotide standards and benzoyl chloride were purchased from Sigma-Aldrich (Poole, Dorset, UK). For both histamine and nucleotide separation, the mobile phase consisted of acetonitrile and HPLC grade water (Philip Harris Scientific, Lichfield, Staffordshire, UK).

2.6. K and related values

K, Ki, H, and G values as indices of freshness quality, were calculated by the procedures described by Saito,

Arai, and Matsuyoshi (1959), Karube et al. (1984), Luong et al. (1992) and Burns et al. (1985), respectively. In this study, the K, Ki, H- and G-values, were each expressed as a percentage; the formulas used were as follows:

$$\text{K-value (\%)} = \left[(\text{Hx} + \text{HxR})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Hx} + \text{HxR})\right] \times 100$$

$$\text{Ki-value (\%)} = [(\text{Hx} + \text{HxR})/(\text{IMP} + \text{Hx} + \text{HxR})] \times 100$$

$$\text{H-value (\%)} = [(\text{Hx})/(\text{IMP} + \text{Hx} + \text{HxR})] \times 100$$

$$\text{G-value (\%)} = [(\text{Hx} + \text{HxR})/(\text{AMP} + \text{IMP} + \text{HxR})] \times 100$$

2.7. Microbiological analysis

Samples were taken to estimate total viable counts and histamine forming-bacteria from each of three different fish stored in air and in VP and in MAP under sterile conditions.

2.8. Total viable count (TVC)

Fish muscle (10 g) was mixed with 90 ml of Ringer solution and then stomached for 3 min. Further decimal dilutions were made and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar (Oxoid) plates in triplicate. They were then incubated for 2 days at 30 °C.

2.9. Histamine-forming bacteria (HFB)

Fish muscle (5 g) was mixed with 45 ml of artificial sea water (50%) for HFB and then homogenised for 3 mins using a stomacher. Serial dilutions of each sample were prepared using 50% artificial sea water (Lyman & Fleming, 1940). Aliquots (0.1 ml) were spread in triplicate over the Niven's medium plates, which were then incubated for 2 days at 30 °C. Purple colonies (surrounded by a purple halo on a yellowish background) were counted for each plate using a colony counter.

2.10. 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 (2000b) 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.

2.11. Statistical analysis

For data analysis, the Student *t*-test, standard deviation and coefficient of variance were used. 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. Proximate analysis

Table 1 shows the compositions of the constituents in sardines used in these studies and their standard deviations and coefficients of variance. Burt and Hardy (1992) reported that the chemical composition of sardines was 57–78% water, 17–21% protein, and 1–21% lipid. The variation in the chemical composition of sardines is closely related to nutrition, living area, fish size, catching season, seasonal and sexual variations as well as other environmental conditions (Pacheco-Aguilar, Lugo-Sanchez, & Robles-Burgueno, 2000). Variation in chemical composition, due to the factors pointed out above, might lead to changes in attributes, including taste, odour, texture, colour, and surface appearance, which control the acceptability of fish as food (Flick & Martin, 1992; Love, 1970).

3.2. Sensory evaluation

The pattern of increase in the demerit score from day 0 to day 15, for sardines kept under three different storage conditions, is shown in Fig. 1. The rate of increase of demerit points is fairly linear with storage time in three cases. Significant differences ($P < 0.05$) were found in the level of demerit points between sardine held in air and in VP, particularly in MAP.

The observed shelf life of sardines was found to be 12 days (demerit score: 19) in MAP, 9 days (demerit score: 17–18) in VP and 3 days (demerit score: 16–17) for fish stored in air. In a previous study, the shelf life of herring stored in a 60/40:CO_2/N_2 gas mixture was found to be

Table 1
Chemical composition of sardine[a]

Proximate composition	% Mean value	S.D.	%CV
Protein content	17.8	0.87	4.89
Lipid content	12.2	0.50	4.13
Moisture content	67.7	2.10	4.09
Ash content	1.1	0.05	5.36

[a] Data are expressed as mean value of five samples.

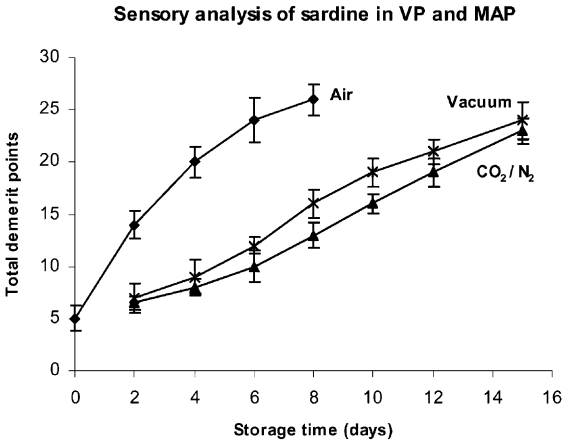

Fig. 1. Changes of sensory values of sardines stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of two determinations for each sampling. Bars represent the standard deviation.

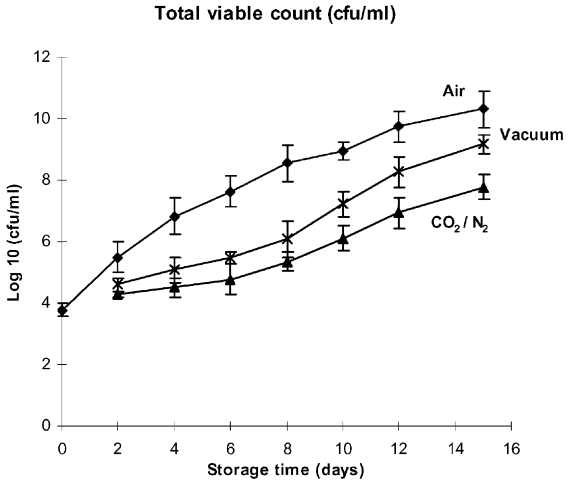

Fig. 2. Total viable counts (cfu/ml) in sardine stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling. Bars represent the standard deviation.

10 days in MAP and 8 days in VP at 2±2 °C (Özogul et al., 2000b). Dhananjaya and Stroud (1994) reported that the MAP herring stored in a 60/40:CO_2/N_2 gas mixture was still acceptable after 14 days, although the control sample was barely acceptable after 12 days of storage.

Clingman and Hooper (1986) found that fresh fish products stored under VP had an overall increase of shelf life of 7 days over aerobically stored fish. In this present study, VP and MAP did significantly extend the sensory shelf life of sardines as compared to storage in air. However, off-odours and drip losses in MAP and VP lowered the sensory quality of the sardines. Cann et al. (1983) indicated that sensory evaluation limited the MAP shelf life of herring to 8 days whereas 13 days was obtained with VP. Their results differed from those of our experiment, in which the shelf life of sardine in MAP was found to be longer than that of sardines stored in VP. Randell, Hattula, and Ahvenainen (1997) observed that the sensory quality of both trout and herring fillets deteriorated faster in over-wrap and vacuum packages than modified atmosphere packages.

3.3. Microbiological assessment

3.3.1. Total viable count (TVC)

Fig. 2 shows total viable counts in sardines stored in air, in VP and in MAP at 4 °C. Bacteria grew most quickly in sardine stored in air, followed by those in VP and the lowest counts were with MAP where the log phase was apparently extended. One of the major mechanisms of MAP and VP techniques is to change the level of oxygen in a food environment so as to have an effect on the growth of different groups of microorganisms. The removal of O_2 is more important than the inclusion of high CO_2 content in the pack, due to oxidation of fat. Aerobic microorganisms are generally sensitive to CO_2; therefore, MAP delays the spoilage of fish. Huss (1972) indicated that CO_2 has an important effect on microbial growth, exerting a selective inhibitory action. To achieve microbiological benefits, the storage temperature of MAP products should be as low as possible, since solubility of CO_2 decreases with an increase in temperature (Daniels, Krishnamurth, & Rizvi, 1985). Pastoriza, Sampedro, Herrera, and Cabo (1996) reported that significant differences ($P<0.05$) were found between control (air) and MAP-stored samples in terms bacterial counts. In the present study, significant differences ($P<0.05$) were observed between samples kept in air and in MAP.

When the aerobic plate count reaches 10^6 cfu (colony forming units) per gramme or millilitre in a food product, it is assumed to be at, or near, spoilage. El-Marrakchi, Bennour, Bouchaiti, Hamma, and Tagafait (1990) reported that initial TVC of iced sardines was 3.16×10^2 cfu/g, reaching the limit counts of 10^6–10^7 cfu/g at day 9, while the counts exceeded these limits within 24 h at ambient temperature. In this study, the limit of acceptability (10^6 cfu/g) in terms of total viable count was 3 days for sardines stored in air, 8 days for VP, and 10 days for MAP. The result obtained from sensory evaluation, after VP and MAP treatment, showed a longer shelf life when compared with microbiological assessment.

3.3.2. Histamine-forming bacteria (HFB)

Changes in the number of histamine-forming bacteria (HFB) of sardines stored in air, in VP and MAP at 4 °C are shown in Fig. 3. HFB increased in all treatments with increasing storage time. Lowest HFB were obtained from sardines stored under MAP, indicating that the presence of the CO_2 in the pack inhibited the growth of microorganisms, which resulted in preventing of spoilage and extending of shelf life of the fish. Sato,

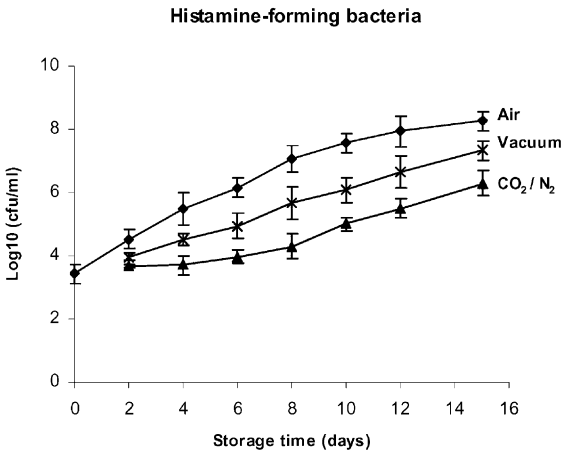

Fig. 3. Histamine-forming bacteria (HFB) in sardines stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling. Bars represent the standard deviation.

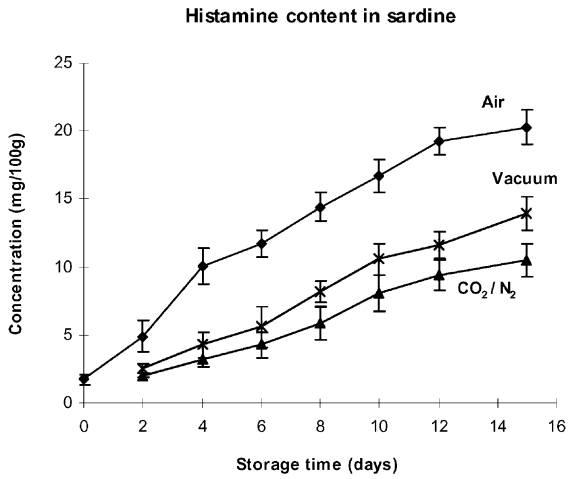

Fig. 4. Histamine content of sardines stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling. Bars represent the standard deviation.

Fujii, Masuda, and Okuzumi (1994) reported that the histamine content of mackerel, stored at 5 °C, increased rapidly when the number of bacteria reached above 10^6 cells/g. Histamine-forming bacterial species and strains differ considerably in amounts of histamine formation, and the type of spoilage bacteria present depends on an aquatic environment (Lopez-Sabater, Rodriguez-Jerez, Hernandez-Herrero, & Mora-Ventura, 1994; Silva, Da Ponte, & Enes Dapkevicius, 1998).

The formation of high concentrations of histamine in fish products can be fairly rapid and depends on the number of microorganisms present (Ababouch, Afilal, Rhafiri, & Busta, 1991; Pacheco-Aguilar, Lugo-Sanchez, Villegas-Ozuna, & Robles-Burgueno, 1998). Enteric bacteria, especially *Morganella morganii*, certain strains of *Klebsiella pneumoniae*, and a few strains of *Hafnia alvei* are the most prolific histamine formers in fish when they are held at temperatures above 4 °C (Stratton & Taylor, 1991) and are the most commonly related to scombrotoxic fish (Taylor & Speckhard, 1983).

3.4. Chemical analysis

3.4.1. Histamine analysis

The changes in histamine content in the muscle of sardines stored in air, in VP, and in MAP at 4 °C are shown in Fig. 4. The concentration of histamine increased and its level reached more than 20 mg/100 g for air treatment, 13 mg/100 g for VP and 10 mg/100 g for MAP. Histamine content did not develop beyond the limit (20 mg/100 g) set by the EU (EEC, 1991) except for fish stored in air. No significant differences ($P > 0.05$) were found between MAP and VP-treated fish in terms of histamine concentration throughout the storage period. Generally, histamine accumulation in sardines stored in VP is significantly lower than in herring stored in air, since bacterial growth is inhibited in VP. Aerobic flora cannot grow in this type of packaging due to the exclusion of O_2.

Histamine in herring was produced more slowly in MAP than in VP at 2±2 °C (Özogul et al., 2002b) and at 5 and 10 °C (Cann et al., 1983). In the present study, the production of histamine was also slower in sardine stored in MAP than sardines stored in VP. Watts and Brown (1982) found that CO_2 atmosphere did not stimulate histamine production in Pacific mackerel, and histamine levels remained lower in CO_2 than air. At the time of rejection, the contents of histamine in herring and mackerel stored in VP at 2 °C were 42 and 43 ppm, respectively (Klausen & Lund, 1986).

The rate of histamine development was much greater at an ambient temperature, and icing significantly reduced or totally inhibited histamine accumulation in sardine (*S. pilchardus*) muscles (Ababouch, Souibri, Rhaliby, Ouadhi, Battal, & Busta, 1996). Pacheco-Aguilar et al. (2000) found a very small amount of histamine (0.0018 mg/100 g) in Monterey sardine (*Sardinops sagax caerulea*) muscle in ice. The content of histamine in sardine stored in air at 4 °C was found to be less than 10 mg/100 g at the last day of sensory acceptability. El Marrakchi et al. (1990) reported that the amount of histamine in sardine (*S. pilchardus*) flesh at the time of rejection (12 days) in ice was 16.2 mg/100 g.

3.4.2. K and related values

The freshness indicators, namely, K, Ki, H, and G values, of sardines were calculated from the concentrations of nucleotide over the 15 days of storage. The increases in the pattern of K-, Ki-, H- and G-values for sardines held under three storage conditions are shown in Figs. 5–8, respectively. Freshness or spoilage indicators related to the breakdown of nucleotides are based

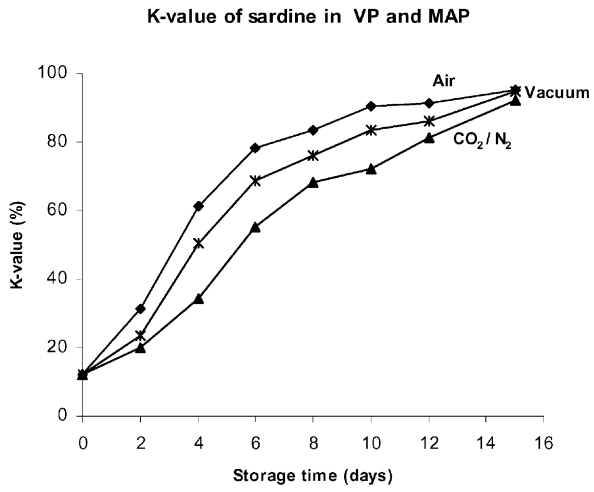

Fig. 5. Mean K-values of sardine stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling.

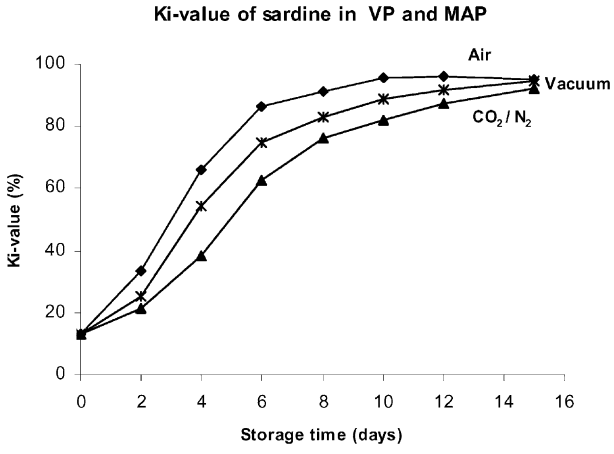

Fig. 6. Mean Ki-values of sardine stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling.

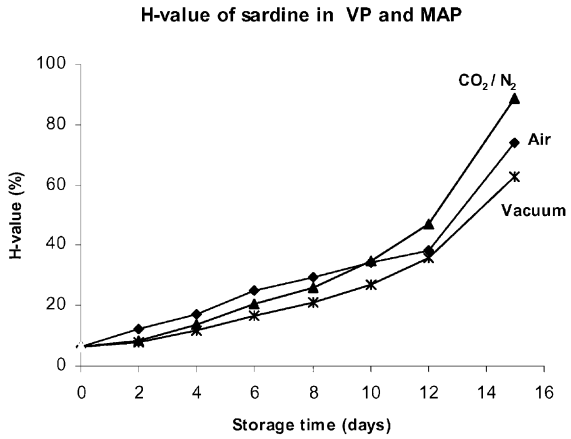

Fig. 7. Mean H-values of sardine stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling.

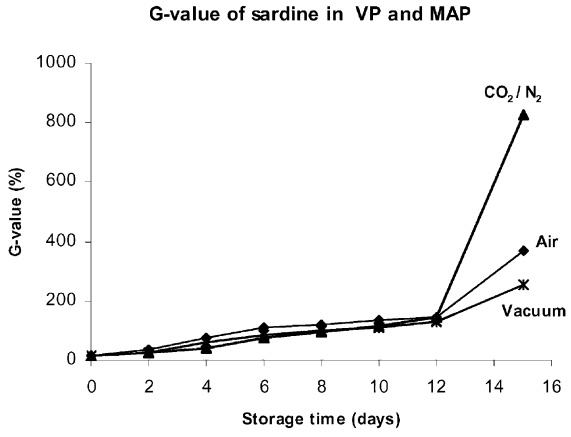

Fig. 8. Mean G-values of sardine stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling.

on the autolysis of ATP in the muscle. The rapid rise of the K-value is entirely due to the sharp decline of IMP in the fish flesh. The loss of IMP through degradation to HxR and Hx would cause a loss of fresh fish desirable compounds. The K-value rose at a fairly moderate rate, reaching over 90% from an initial value of 12% after 15 days of storage. The highest K-values were obtained from sardines kept in air, followed by those in VP. The lowest increase in K-value was obtained from sardine stored in MAP, which was possibly influenced by the presence of the CO_2. This is in agreement with previous studies with herring (Özogul et al., 2000b). There was a significant difference ($P<0.05$) between the treatments stored in air and MAP except before 2 days and after 10 days of storage.

Ki-values increased continuously and reached over 90% from an initial value of 13% during 15 days of storage. Significant differences ($P<0.05$) were observed between sardines held in air and the MAP trial from day 2 to day 10. No significant differences ($P>0.05$) were found in sardines stored in air and VP, and also MAP and VP throughout 15 days of storage. On the day of marginal acceptability, the Ki-value of sardines was 84% for VP and 87% for MAP storage conditions. In vacuum-packed and modified atmosphere-packed herring, the Ki-value on the day of marginal acceptability was about 85 and 84%, respectively (Özogul et al., 2000b). Huynh, Mackey, and Gawley (1992) found a good correlation between sensory fish freshness and the Ki-value (they called it K-value) for sockeye salmon and Pacific herring stored in ice and at 12 °C. However, the Ki-value with some species has been observed to increase very rapidly and then remain constant, although the freshness quality continues to decrease greatly (Greene & Bernatt-Byrne, 1990). Atlantic cod or Pacific cod rapidly accumulated HxR; thus Ki-value is not a suitable index to determine freshness quality for such species (Luong et al., 1992). These fish species have

been observed to rapidly accumulate a large amount of H×R compared with the accumulation of Hx when the level of IMP rapidly decreases.

The initial level of H-value was found to be 6% and reached up to 62% for sardine stored in air, 74% for VP and 88% for MAP at the end of storage time. H-value continuously rose up to 12 days of storage, after which it increased suddenly. The sudden increase of H-value was influenced most strongly by quick IMP decomposition. Unlike K-value and Ki-value, the highest H-values were observed for MAP storage conditions, due to a rapid decrease of inosine. No significant differences ($P > 0.05$) were observed amongst storage conditions throughout 15 days of storage. Luong et al. (1992) reported that the H-value of iced Pacific cod has been observed to immediately begin to increase quite steadily and thus also to be much superior to the Ki-value.

G-values of sardines kept under all storage conditions increased very slowly up to 12 days in contrast to a large increase towards to the end of the storage period. The G-value was found to be 143% for MAP when the fish was not considered acceptable by the panellists. The results obtained from this work, regarding the G-value, revealed that it is of no use as a freshness index for sardines. However, the G value has been reported to be much superior to the Ki-value for some species (such as Atlantic cod), although it has been observed to decrease during the first 2 or 3 days of storage in ice before its subsequent steady rise (Burns et al., 1985).

Using the K-value of 72% as an example, this value would correspond to a shelf-life of about 10 days for sardines stored in MAP. Based on the sensory demerit score, sardine stored in VP and MAP were acceptable up to 9 and 12 days, respectively. These results related to values of K that were 80% for VP and 81% for MAP storage condition, while the K- and Ki-values correlated with the perceived loss of freshness. The individual values did not accurately reflect product acceptability under different storage conditions. No significant differences were found between K-value and Ki-value, since ATP, ADP, and AMP were converted to IMP quickly at an early stage of storage.

3.4.3. TMA and TVB-N

The concentrations of TMA present in the muscle tissue of sardines stored in air, in VP and in MAP at 4 °C are shown in Fig. 9. The highest concentration of TMA was obtained from sardines stored in air, followed by sardines stored in VP and the lowest in MAP. This is probably because MAP inhibits bacterial growth and reduces the formation of TMA, resulting in an extension of shelf life of fish. MAP, in general, had an inhibitory effect on the growth of the microflora and limited inhibition of the production of TVB and TMA (Debevere & Boskou, 1996). The concentrations of TMA-N in numerous fatty fish never reached the limit of 5 mg

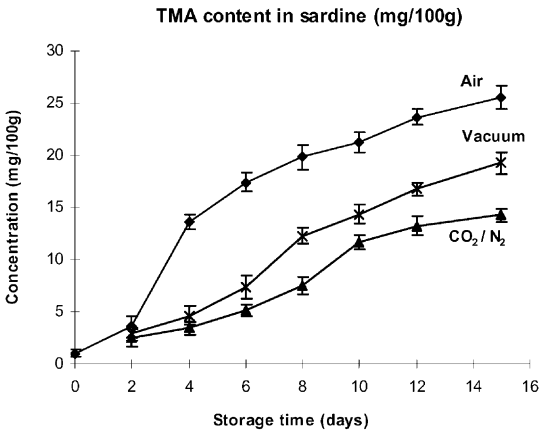

Fig. 9. TMA content of sardines stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling. Bars represent the standard deviation.

TMA-N/100 g, although the rejection limit in fish flesh is usually 5–10 mg TMA-N/100 g (23–46 mg TMA/100 g) (Sikorski, Kolakowska, & Burt, 1990). Nevertheless, this limit cannot be applied to sardines stored under MAP and VP since fish were spoiled before reaching this level. This could be because sardine is a fatty fish. The concentration of TMA was found to be 13.5 mg in herring kept in MAP (60/40:CO_2/N_2) for 10 days at 2±2 °C (Özogul et al., 2002b) and 10.4 mg/100 g in herring fillets stored in a MAP with the same gas mixture for 11 days at 0 °C (Cann et al., 1983). The results obtained from this experiment related to TMA production show that significant differences ($P < 0.05$) were discovered between sardines held in air and VP and, especially, MAP treatments after 4 days of storage.

The level of TMA was typically around 10–15 mg TMA-N/100 g in aerobically stored fresh fish rejected by sensory panels (Dalgaard, Gram, & Huss, 1993). Pastoriza et al. (1996) reported that TMA production was significantly ($P < 0.05$) reduced by storage under CO_2 (MAP). The limit of acceptability for sardine was found to be 5–10 mg TMA-N/100 g of samples according to comparison of sensory and chemical data (Ababouch et al., 1996).

TVB-N content of sardines stored in air, VP and MAP at 4 °C is shown in Fig. 10. At the beginning of storage, the TVB-N value was 5 mg/100 g flesh for sardines stored in air. The release of total volatile bases increased up to 15 mg/100 g for sardine stored in air, 19 mg/100 g for sardine in VP and 17 mg/100 g in MAP at the last day of sensory acceptability for each storage condition. Fraser and Sumar (1998) indicated that bacterial catabolism of amino acids in fish muscle results in the accumulation of ammonia and other volatile bases. The statistical analysis of the TVB-N data showed that significant differences ($P < 0.05$) were found between sardine stored in air and VP, and particularly MAP, after 4 days of storage. However, there were no sig-

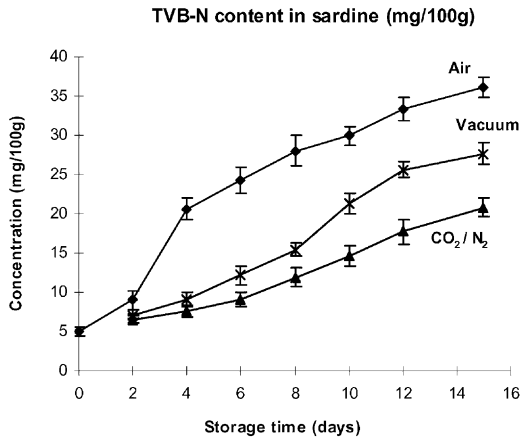

Fig. 10. TVB-N content of sardines stored in air, in VP and in MAP at 4 °C. Each point shown is the mean value of three determinations for each sampling. Bars represent the standard deviation.

nificant differences ($P > 0.05$) between the TVB-N values at different stages of storage of sardines in VP and MAP.

As for many fish species, the formation of TVB-N increased with time of storage. When the TVC had reached 10^6 cfu/g, the TVB-N content was found to be approximately 15 mg/100 g muscle for all storage conditions. The more rapid increase of TVB-N at higher microbial numbers indicated the stage of substantial spoilage of the fish. Ababouch et al. (1996) reported that the limit of acceptability for sardine was 25–35 mg TVB-N/100 g of flesh.

4. Conclusions

Storage of sardines under MAP conditions decreased the production of ATP derivatives, inhibited bacterial growth, reduced the formation histamine, TMA and TVB-N, and extended the shelf life. Shelf life extension was 4-fold under MAP and 3-fold under VP.

The freshness indicators, K- and Ki-values, are superior to H-value and G-value and provided useful freshness indices for sardines in MAP and VP. However, they did not correlate well with sensory assessment. G-value did not give a good linearity with sensory evaluation; thus this freshness index cannot be usefully used for sardine, since, although freshness quality continued to decline, G-value increased very slowly.

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