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Chemical, microbiological and sensory evaluation of Atlantic herring (*Clupea harengus*) stored in ice, modified atmosphere and vacuum pack

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Abstract

The effects of modified atmosphere and vacuum packing on *K* values, microbial and sensory changes in herring (2 days in ice post capture) when stored at 2±2°C are investigated for up to 16 days. Although chemical and microbiological analyses indicated that CO_2 and vacuum packing prolonged the shelf life of herring, compared with storage in ice, sensory analysis showed that the extension of shelf life was only with MAP (10 days) and VP (8 days). It was also found that 60% CO_2 treatments showed lower *K* values compared to those observed for aerobically held fish. The results showed significant ($P < 0.05$) differences between ice and MAP storage conditions. In addition, CO_2 decreased the formation of Hx compared to aerobically and vacuum-held samples.

Keywords: Herring; Storage life; MAP; VP; *K* value

1. Introduction

Many chemical methods have been suggested as indices of deterioration of fish quality during storage. These include TMA (Gill, 1990; Tozawa, Enokihara & Amano, 1971), DMA (Castell, Neal & Dale, 1973; Oehlenschlager, 1992), Hx (Jacober & Rand, 1982; Jones, 1965), IMP (Hattula, Kievaara & Moran, 1993) and *K* value (Ehira & Uchiyama, 1987; Spinelli, Eklund & Miyauchi, 1964). Among these methods, the concentrations of ATP and its breakdown products are considered to be the most reliable and useful indicators (Karube, Matsuoka, Suzuki, Watanabe & Toyama, 1984). Although the pattern of ATP breakdown varies between species, it has been used to determine freshness in a variety of fish when expressed as the *K* value (Hondumrankil & Silva, 1994). After fish have been caught, the levels of adenosine triphosphate decrease and most of the adenosine nucleotides are degraded to inosine monophosphate (IMP) in 1–3 days. As the degradation continues, inosine and then Hx will be produced. Hx has a bitter flavour whereas IMP is desirable as a flavour component in fresh fish.

There has been much research on the use of single ATP derivatives or combinations as an index of freshness in variety of fish. The concentration of Hx has been proposed as an index of freshness of fish but it does not always correlate with sensory quality (Jones, Murray, Livingston & Murray, 1964). However, the *K* value proposed by Saito, Arai and Matsuyoshi (1959), includes intermediate breakdown products. Since adenosine disappears very quickly, Karube et al. (1984) proposed the K_i value which excludes ATP, ADP and AMP. *K* value varies within species of fish (Murata & Sakaguchi, 1986; Ryder, Buisson, Scott & Fletcher, 1984). In addition, *H* values have been described by Luong, Male, Masson and Nguyen (1992) as an index of freshness quality.

Modified atmosphere packing (MAP) with refrigeration has the ability to extend the shelf life of seafood (Banks, Nickelson & Finne, 1980; Brown, Albright, Watts, Heyer, Spruce & Price, 1980; Church, 1998; Davis, 1990). MAP has been shown to extend shelf life and decrease the rate of degradation of ATP in fish. The effects of MAP on seafood have been reviewed extensively (Davis, 1993; Farber, 1991; Reddy, Armstrong,

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Rhodehamel & Kauter, 1992; Stammen, Gerdes & Caporaso, 1990) but little information is available on storage of herring in modified atmosphere packing.

The aim of the present study was to compare the quality of Atlantic herring stored at 2±2°C in boxes without ice, in vacuum packaging (VP) and modified atmosphere packaging (MAP) using herring stored in ice as a reference sample in terms of chemical, microbiological and sensory analysis.

2. Materials and methods

2.1. Packaging and storage of herring

Atlantic herring (*Clupea harengus)* were obtained from Mallaig in Scotland, gutted and divided into four lots. The fish stored in ice were 2 days post capture on arrival at the laboratory. One lot was iced in boxes as reference samples and another stored in a box without ice at chill temperature (2±2°C). The remaining two lots were placed in nylon-polyethylene pouches (30 × 35 cm), the third lot vacuum packed and the fourth lot gas packed in a Multivac model A 300 vacuum-packaging machine (Bury, Lancashire, UK). The O_2 transmission rate of pouches was 47 cc/m^2 24 h. The gas ratio was 60% CO_2 and 40% N_2, typical for packing fatty fish in MAP (Cann et al., 1983). The final gas/samples ratio in all pouches was about 2:1 (v/w) for MAP conditions. All samples were stored in a chill room (2±2°C). Data were obtained using three batches of herring. Duplicate samples were taken for chemical analysis from each of two different fish for each batch kept under MAP, VP, iced and in chilled box without ice every 2 days throughout the storage period.

2.2. Chemical analysis

ATP and its degradation products were analysed by using a rapid HPLC method (Özogul, Taylor, Quantick & Özogul, in press). The extraction procedure was based on that of Ryder (1985).

2.2.1. Apparatus

The high-performance liquid chromatography (HPLC) system with an intelligent pump (Merck-Hitachi L-6200A) and a diode array detector (Merck-Hitachi L-4500) was used. The separations were performed on a Sphereclone ODS 2 C_{18}, 150×4.60 mm, 5 μm particle diameter column (Phenomenex, Macclesfield, Cheshire, UK), with a matching guard cartridge.

The mobile phase consisted of acetonitrile (Philip Harris Scientific, Lichfield, Staffordshire, UK) and phosphate buffer solutions. The injection volume was 5 μl and detection was monitored at 254 nm.

2.2.2. Nucleotides analysis

K, K_i and H values were calculated by the procedures described by Saito et al. (1959), Karube et al. (1984) and Luong et al. (1992), respectively. In this paper, the K, K_i and H values were expressed as percentages and the formulas used are as follows:

$$\left\{K\,\text{value}\,(\%) = \left[(\text{Hx} + \text{HxR})/\left(\text{ATP} + \text{ADP} + \text{AMP} + \text{Hx} + \text{HxR}\right)\right] \times 100\right\} \quad (3)$$

$$\left\{K\text{i value}\,(\%) = \left[(\text{Hx} + \text{HxR})/(\text{IMP} + \text{Hx} + \text{HxR})\right] \times 100\right\}$$

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

2.3. Microbiological analysis

Duplicate samples were taken to estimate total viable counts from each of two different fish stored under four different storage conditions for each batch. Three batches of herring were used for this experiment. Ten grams of fish muscle were 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 plates in triplicate. They were then incubated for 2 days at 30°C.

2.4. Sensory analysis

For sensory analysis, duplicate samples from each of the four storage conditions were taken at regular intervals for each batch. Three different batches of herring were used for this experiment. Each assessment was carried out by a minimum of six trained panellists. Sensory analysis was assessed using the Tasmanian Food Research Unit scheme (Branch & Vail, 1985) with minor modifications for herring. Table 1 shows the modified Tasmanian Food Research Unit freshness assessment scheme. 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 with a demerit score of 18±2.

3. Results and discussion

3.1. Chemical analysis

Figs. 1–4 illustrate the pattern of nucleotide degradation in the muscle of herring stored at chill temperature

Table 1
Modified Tasmanian Food Research Unit freshness assessment scheme

Score	0	1	2	3
General				
Appearance	Very bright	Bright	Slightly dull	Dull
Skin	Firm	Soft		
Slime	Absent	Slightly slimy	Slimy	Very slimy
Stiffness	Pre-rigor	Rigor	Post-rigor	
Eyes				
Clarity	Clear	Slightly cloudy	Cloudy	
Shape	Normal	Slightly sunken	Sunken	
Iris	Visible	Not visible		
Blood	No blood	Slightly blood	Very bloody	
Gills				
Colour	Characteristic	Slightly dark Slightly faded	Very dark Very faded	
Mucus	Absent	Moderate	Excessive	
Smell	Fresh oily, metallic seaweed	Fishy	Stale	Spoilt
Belly cavity				
Stains	Opalescent	Greyish	Yellow–brown	
Blood	Red	Dark red	Brown	
Total demerit points[a]				

[a] Sum of score is minimum 0 and maximum 27.

in a box without ice, in ice, VP and MAP, respectively. Since the fish were 2 days old, the values for ATP, ADP and AMP were low (< 0.4 μg/g) and then decreased gradually under the four conditions. The content of IMP decreased most rapidly in fish muscle in no ice and most slowly in MAP. This result is an agreement with the pattern for Pacific herring kept in ice studied by Huynh, Mackey and Gawley (1992).

As the degradation of nucleotides progressed, the levels of inosine and hypoxanthine (Hx) increased. The concentration of Hx for herring stored in VP, ice and boxes without ice increased during the early stage of storage, reached a peak and then decreased. The Hx content of herring held in ice and VP increased more rapidly than herring held under carbon dioxide (60%) which increased steadily to 1.9 μmol/g after 16 days, indicating that the presence of carbon dioxide influenced the accumulation of Hx. This result corresponds to that of Dhananjaya and Stroud (1994) who found that lower Hx contents were obtained in CO_2 packs than in iced herring. In addition, Warthesen, Waletzko and Busta (1980) reported that Hx contents of fish stored in 100% CO_2 were lower than in products stored at lower CO_2 concentrations.

Initial K values for herring stored in boxes with no ice, with ice, VP and in MAP were found to be 23%, and increased (Fig. 5). The IMP dropped rapidly, resulting in an increase in K value with increasing storage time and there was a significant difference ($P < 0.05$) between the treatment in ice and MAP

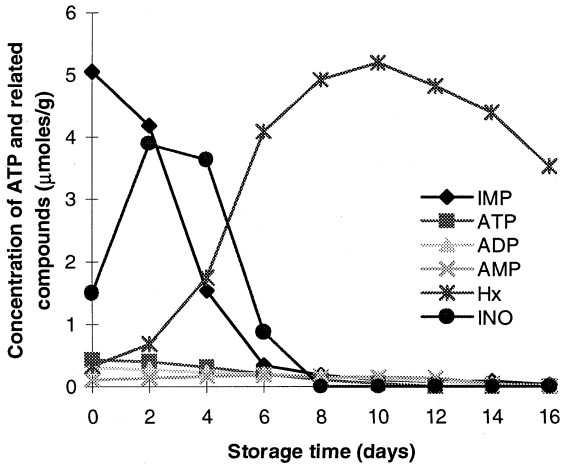

Fig. 1. Nucleotide degradation of herring stored in boxes without ice at 2±2°C.

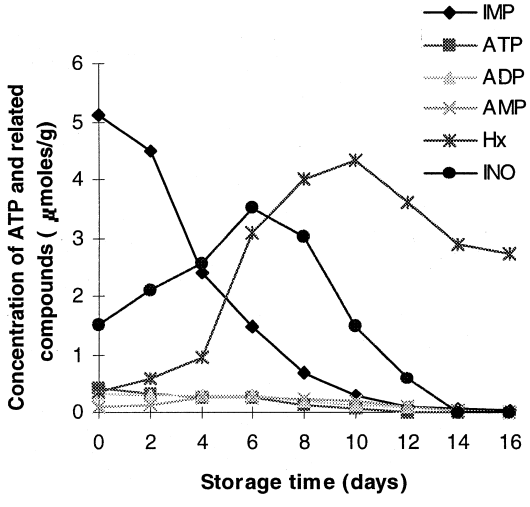

Fig. 2. Nucleotide degradation of herring stored in ice.

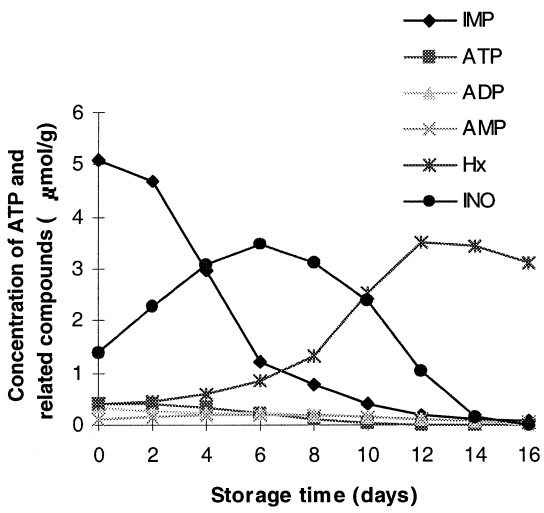

Fig. 3. Nucleotide degradation of herring stored in VP at 2±2°C.

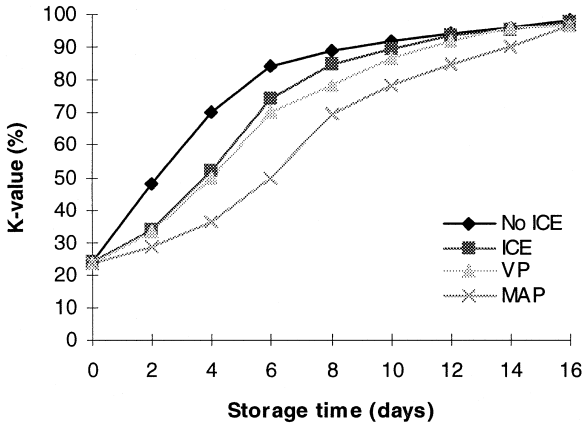

Fig. 5. Mean K values of herring stored in ice, VP, MAP (60% CO_2, 40% N_2) and in boxes without ice at 2±2°C.

storage of herring, the lowest K value being obtained from herring in MAP. This differs from the results of Lopez-Galvez, de la Hoz, Blanco and Ordonez (1998) with sole fillets who reported no effect of the atmosphere on the K values. Reddy, Roman, Villanueva, Solomon, Kautter and Rhodehamel, (1997) also found that, at 4, 8 and 16°C storage, K values of MAP-stored fillets of catfish increased gradually during early and middle storage time and decreased towards end of storage period with sensory spoilage, indicating no relationship between sensory spoilage and K value.

Hx concentration differed within samples during the storage period but increased with storage time. This is in agreement with previous studies (Özogul et al., in press). Rockfish and salmon in air showed increased Hx concentration over time (Brown et al., 1980; Parkin, Wells & Brown, 1981). However, they indicated that the values for MAP were inconsistent. The same results for rockfish and white fish were found by Lindsay, Josephson and Olafsdottir (1986). However, in this experiment, concentration of Hx increased gradually with storage time for herring kept under four different storage conditions and significantly lower in CO_2 than others. This could be attributed to CO_2 inhibiting the formation of Hx.

The increase in the patterns of K, K_i and H values for herring, kept under four different storage conditions (Figs. 6–9) were observed with increase in storage time and significant differences were obtained within different conditions ($P < 0.05$). The lowest values of K, K_i and H were obtained from herring in MAP, followed by VP, ice and in box without ice. K values were found to be linearly related to K_i values throughout the storage period. However, H values of herring have been observed to increase very slowly during the storage period (except for herring stored without ice) compared to K and K_i values, although the freshness quality continued to decrease greatly. It can be inferred that values of K, K_i are superior to the H value and provide useful freshness indices for herring in MAP and VP. However, they do not correlate with sensory assessment.

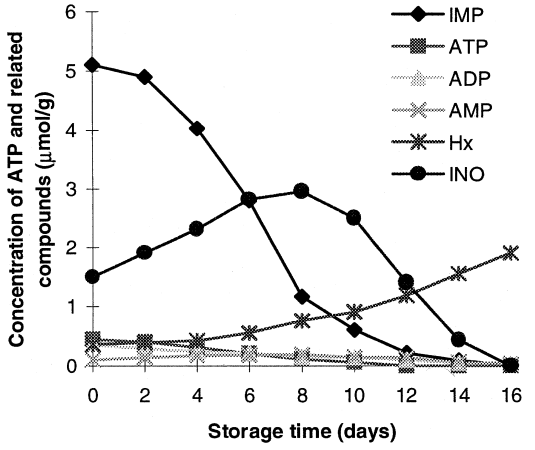

Fig. 4. Nucleotide degradation of herring stored in MAP at 2±2°C.

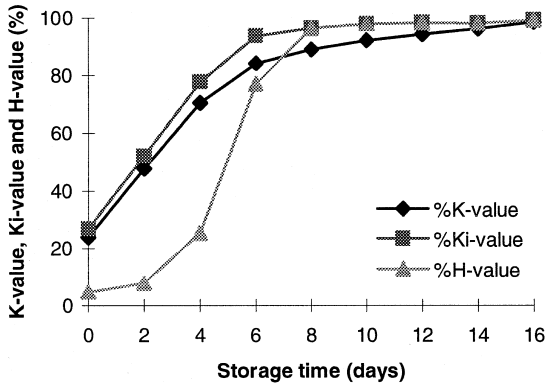

Fig. 6. Mean K, K_i and H values of herring stored in boxes without ice at 2±2°C.

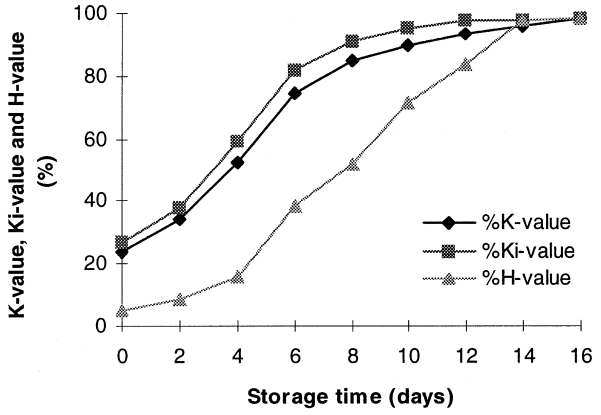

Fig. 7. Mean K, K_i and H values of herring stored in ice.

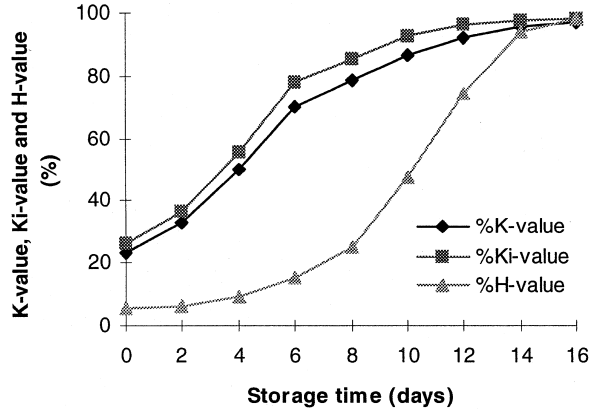

Fig. 8. Mean K values of herring stored in VP.

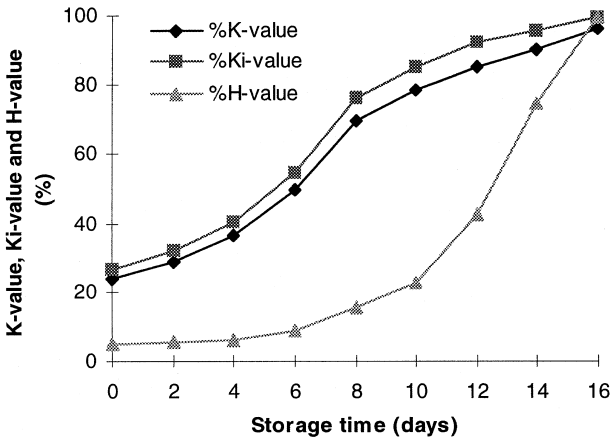

Fig. 9. Mean K, K_i and H values of herring stored in MAP (60% CO_2, 40% N_2).

3.2. Microbiological quality

The initial quality of fish used in this study was good, as indicated by a low initial number of bacteria (10^4 cfu/g) before fish were subjected to the different storage conditions. Bacteria grew most quickly in herring kept in a box without ice, followed by those in ice and vacuum-packing (Fig. 10). Lowest counts were with

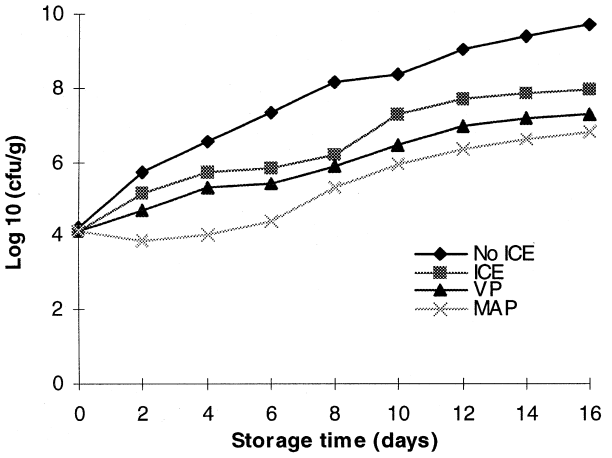

Fig. 10. Total viable count in herring stored in ice, VP, MAP and in boxes without ice.

MAP where the log phase was apparently extended. It has been reported that CO_2 has an important effect on microbial growth, exerting a selective inhibitory action (Huss, 1972). Aerobic micro-organisms are generally sensitive to CO_2, therefore MAP delays the spoilage of fish and other seafood. Molin, Stenstrom and Ternstrom (1983) demonstrated that refrigeration storage in 100% CO_2 prolonged the microbiological shelf life of herring fillets and that the flora were directed towards *Lactobacillus* spp. Initial bacterial population, gas/fish ratio, and packaging materials are important factors affecting shelf life of fish in packages. To achieve microbiological benefit, the storage temperature of MAP product should be as low as possible since solubility of CO_2 decreases with increase in temperature (Daniel, Krishnamurth & Rizvi, 1985).

It has also been reported that CO_2 has been shown to delay spoilage of fresh seafood by inhibiting psychrotopic, aerobic and Gram negative bacteria (Banks et al., 1980; Brown et al., 1980; Finne, 1982; Layrisse & Matches, 1984).

3.3. Sensory analysis

Sensory assessment of herring revealed differences among the different treatments. When CO_2 and VP bags were opened, fish initially had no odour and then fishy odour but this changed to off odour as storage period extended. The appearance of herring was poorer in vacuum- and gas-packaging than in ice due to the excessive drip.

The observed shelf life of herring, as determined by panellists who indicated that the fish were not acceptable, was longest in MAP (10 days), followed by VP (8 days) and ice (8 days), and least with no ice (less than 4 days). The storage life of fish is affected by the initial microbial load of the fish, storage temperature and packaging methods (Church, 1998).

Although MAP extended the shelf life of herring, volatile off-odours, which accumulated during the storage period of fish, limited shelf life of fish in terms of sensory evaluation. Vacuum-packing did not significantly extend the sensory shelf life of herring as compared to storage in ice, whereas MAP did extend the shelf life. However, off-odours and drip loss in MAP and VP lowered the sensory quality of herring. Cann, Smith and Houston (1983) indicated that sensory evaluation limited the MAP shelf life of herring to 8 days whereas 13 days was obtained with vacuum packages. They found that chemical and bacterial analyses of herring in MAP indicated a longer shelf life but cooked flavour was found to be unacceptable after 8 days in MAP. In this experiment, 10 days storage for herring in MAP was found in relation to sensory evaluation whereas 8 days for herring in vacuum-packing were obtained.

Unacceptable sensory quality of vacuum- and modified atmosphere-packaged herring occurred 8 days for vacuum and 10 days for MAP at which time *K* values were greater than 78%. Although the herring in VP and MAP showed shorter shelf life with reference to sensory analysis, there was an increase in *K* value with an increase in storage time, suggesting that *K* value provided an useful indicator for freshness in herring stored in VP and MAP.

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