Original Article

Modified atmosphere packaging of fish salad

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ABSTRACT: The shelf life of fish salads in modified atmosphere was studied and compared with those of air-packaged (control) products. Samples were evaluated at 0 days, 7 days, and 14 days at 4°C, respectively. The sensory quality of modified-atmosphere packaging (MAP) groups was significantly ($P \leq 0.05$) higher than the control group. Control packages were below the limit of acceptability at the seventh day of storage. However, MAP samples ($O_2:CO_2:N_2 = 1:7:12$ and $CO_2:N_2 = 3:7$) were not rejected until the 14th day of storage. Modified-atmosphere packaging decreased the microbiological activity and the sensory score changes, as well as extending the shelf life of samples by approximately 50%.

KEY WORDS: fish salad, modified-atmosphere packaging, shelf life.

INTRODUCTION

Fish is a very perishable food, having a short shelf life. Modified-atmosphere packaging (MAP) is successful in extending the shelf life of freshwater fish and fish-based products. This technology has been used for retail outlets and catering establishments in recent years. The packaging of food products in high-barrier materials and changing the gaseous environment to slow down respiration rates is a process called modified-atmosphere packaging. This system slows the respiration rates, reduces microbiological growth, and retards enzymatic spoilage. Many studies have shown that, MAP systems are effective on fish-like trout, salmon, croaker, sardine, or jack mackerel. Carbon dioxide, oxygen and nitrogen are the more common gases used in MA systems. Meats exhibit similar spoilage patterns to seafoods during MA storage, but higher and more effective CO$_2$ concentrations can be used for seafoods. Carbon dioxide is used to maintain the fungistatic and bacteriostatic, properties, whereby levels of 20–40% of this gas are used in MAP. Gas levels higher than 40% might cause the packaging to collapse while levels at less than 20% may not inhibit microorganism growth satisfactorily. Higher levels of CO$_2$ with reduced levels of oxygen are used for fatty or smoked fish to slow down the development of oxidative rancidity. Optimum gas ratio must be specific to the species of fish or fish products being packaged and it must be prepared for all. Factors that must be considered when determining the ideal gas concentration are: (i) the gas to fish ratio; (ii) the fish species; (iii) the packaging method; (iv) initial microbial contamination; and (v) temperature. For example, low temperatures inhibit the growth of Clostridium botulinum because the packaging method prevents contamination and maintains the atmosphere, while a specific gas mixture retards the growth of spoilage bacteria.

The shelf life of packaged fish salad in MA was studied. Analyses were done on fish materials only. The aim was to determine the effect of gas mixtures on the sensory evaluation, biochemical alterations, and bacterial growth of fish salads.

MATERIALS AND METHODS

Experimental design

Rainbow trout Oncorhynchus mykiss were gutted, cooked in boiled water for 15 min, and their bones, heads and skins removed. The cooked flesh was
then chopped and mixed with boiled vegetables (potatoes, carrots and peas) and served onto plastic plates (60 g of cooked flesh, 20 g of potatoes, 20 g of carrots, and 20 g of peas). The plates were wrapped with a gas-barrier film (O₂ permeability, 6.89 mL/m²; CO₂, 5.42 mL/m²; N₂, 2.48 mL/m² at 4°C; and vapor permeability, 7.86 mL/m² at 37.8 ± 1°C, 90% ± 2RH g/m² days.atm).

Products were divided into three groups and stored at 4°C. Control samples were packaged under atmospheric conditions. For group A, the fish salads were stored in a O₂ : CO₂ : N₂ gas mixture of 1 : 7 : 12; whereas, group B was stored in a CO₂ : N₂ gas mixture of 3 : 7. Carbon dioxide is the most effective gas in MAP systems; however, higher values of CO₂ would cause higher levels of lactic acid to form in the muscle of fish. This might be the reason for the increased texture, sour taste, and leaking of fish oil. We used O₂ concentrations of 5% and 0% in the present study because higher values can cause the discoloration and oxidation of fish material. As is commonly known, N₂ is an inert gas, and its concentration depends on the amount of the other gases present in the package. A Multivac Sepp Haggenmüller KG D-8941 Wolfertschwenden gas mixer (Multivac GMBH, Wolfertschwenden, Germany) and a Kramer Grebe vacuum apparatus (Kramer Grebe GMBH, Biedenkopf-Wallau, Germany) were used for packaging and flushing.

At 7-day intervals, three packs were taken from each group to be analyzed. The pH and moisture content of fish were studied, and the microbiological and biochemical analyses were continued until the samples had spoiled according to the sensory evaluation score.

Sensory evaluation

Five judges assessed the sensory properties of samples and a hedonic scale was used for sensory analyses. General appearance, texture, taste and smell were used as criteria for acceptability. The mean value of these criteria was used as the sensory evaluation score, whereby a score of 9 was the highest score for the panel testing and a score <4 was considered as spoiled.

Textural properties

An Instron testing machine (model 1140; Instron Ltd, Highwycombe Bucks, UK) equipped with Kramer shear (2830-018) and Plunger assembly (2830-010) was used for texture analyses. The instron was operated with a 500 kg loaded cell and the load range was 50%. For each packaging group, four fish (25 g each, boiled and chopped into 1.5 cm × 1.5 cm × 1.5 cm cubes) were crushed in the instron testing machine at 16°C to evaluate texture.

Color

The color of the fish salad samples was determined with the help of a Minolta chroma meter (model CR 300; Minolta, Osaka, Japan). L* (brightness), a* (+a, red; −a, green) and b* (+b, yellow; −b, blue) values were measured.

Moisture and pH analyses

Moisture content was determined according to the AOAC method, while pH measurements were done using a pH meter (model 632; Metrohm, Filderstadt, Switzerland)

Headspace gases

Headspace concentrations of O₂ and CO₂ were measured immediately after packaging using a Servomex oxygen analyzer 574 and a Servomex infra-red gas analyzer PA (404 SVS; Servomex, Sussex, UK; range 0–100% CO₂) every 7 days.

Biochemical analyses

For total volatile bases nitrogen (TVB-N) estimation, the vapor distillation method was used. Fish samples were boiled in distilled water with 1 g of MgO and the basic vapor components trooped with 0.1 N HCl. Hydrochloric acid was titrated with 0.1 N NaOH and the TVB-N was expressed as mg/100 g fish.

Microbiological properties

The three packages (control, and groups A and B) were opened aseptically to analyse for aerobic plate counts, Staphylococcus aureus, yeasts and molds, coliforms, anaerobics, and Salmonella sp. Fish samples were diluted in peptone water and mixed in a stomacher. Aerobic bacteria were determined by growing cultures in plate count agar (PCA) and incubating for 24 h at 37°C. Violet red bile agar (VRB) was used to determine coliforms, and malt extract agar (MEA) for yeasts and molds. Staphylococcus aureus was estimated by plating in Baird-Parker agar (BP), and anaerobes were determined by differential reinforced clostridial
medium (DRCM). Salmonella sp. were evaluated as either negative or positive.\textsuperscript{18}

**Statistical analysis**

Statistical differences were determined by the method of Renner.\textsuperscript{19}

**RESULTS AND DISCUSSION**

**Sensory evaluation**

The sensory quality of fish salads in the control packages was below the acceptable limit on the seventh day of storage at 4°C (Table 1). The sensory quality of modified atmosphere groups was significantly ($P \leq 0.05$) higher than the control group at the seventh day of storage. Modified atmosphere-packaged samples (groups A and B) were rejected on the 14th day of storage. In a similar study, vegetable salads with herring were packaged in modified atmosphere.\textsuperscript{20} The taste of gas-packaged ($O_2 : CO_2 : N_2 = 1 : 7 : 12$ and $CO_2 : N_2 = 3 : 7$) salads was significantly better than that of air-packaged salads after six days at 4°C. The herring salad of air-packaged samples tasted bitter and was old. There was no clear difference in the taste between the different modified atmospheres.

In a study by Dhananjaya and Stroud, gutted haddock was stored at 0°C, 5°C and 10°C under $CO_2 : N_2 : O_2 = 4 : 3 : 3$. Control and MAP samples spoiled after seven days at 5°C and after four days at 10°C. The MAP samples at 0°C had a longer shelf life than the control samples.\textsuperscript{21} It seems that the storage temperature and the ratio of gases are very important factors in determining the shelf life of MAP products. In the present study, the shelf life of samples were different compared to their study because of: (i) the species of fish; (ii) differences in the storage conditions and gas ratios used; and (iii) the present study used cooked fish samples, whereas they used raw fish samples.

After storing rockfish fillets in MAP, Parkin et al. have reported that the air–MAP samples ($CO_2 : air$ = 4 : 1) were of good quality for at least 13 days of storage.\textsuperscript{22} Their results are fairly similar to the results obtained by the present experiment.

**Textural properties**

Texture analyses were done to determine the effects of gases on textural properties (Table 1). The texture values of samples decreased during storage for the control group and these instrumental values were confirmed by sensory evaluation. There was a significant difference between the control and MAP groups, whereby texture values increased significantly ($P \leq 0.05$) in MAP group A samples (Table 1) due to the production of lactic acid in the fish muscle because of the higher concentrations of CO$_2$ (35%) used in this group. The acidic media denatured the fish proteins and increased the texture value during storage at 4°C. In another study, fish fillets were treated with NaCl solution and CO$_2$.\textsuperscript{23} The mixture of CO$_2$ and O$_2$ hardened the samples, but the CO$_2$ and N$_2$ atmospheres softened them.

**Color properties**

$L^*$ values decreased from 85.56 to 79.97 for the control group, to 80.52 for the A group, and to 76.51 for the B group. $a^*$ Values decreased from 2.12 to 0.52 for the control group, to 0.32 for the A group, and to 1.36 for the B group. $b^*$ Values increased from 14.58 to 15.77 for the control group, to 15.05 for the A group, and to 15.76 for the B group (Table 1). These findings showed that the color of the product turned to yellow, which then darkened and became green when compared to the original

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 0</th>
<th>Control</th>
<th>Day 7</th>
<th>Day 14</th>
<th>MAP (A)</th>
<th>Day 7</th>
<th>Day 14</th>
<th>MAP (B)</th>
<th>Day 7</th>
<th>Day 14</th>
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<tbody>
<tr>
<td>Sensory score</td>
<td>9</td>
<td>3.8</td>
<td>1.0</td>
<td>8.5</td>
<td>2.5</td>
<td>7.0</td>
<td>2.0</td>
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<tr>
<td>Texture (Newton)</td>
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<td>426</td>
<td>795</td>
<td>932</td>
<td>723</td>
<td>762</td>
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<td></td>
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<td>$L^*$</td>
<td>85.56</td>
<td>86.30</td>
<td>79.97</td>
<td>82.94</td>
<td>80.52</td>
<td>78.85</td>
<td>76.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a^*$</td>
<td>2.12</td>
<td>0.57</td>
<td>0.52</td>
<td>0.23</td>
<td>0.32</td>
<td>1.84</td>
<td>1.36</td>
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<tr>
<td>$b^*$</td>
<td>14.58</td>
<td>15.98</td>
<td>15.77</td>
<td>14.76</td>
<td>15.05</td>
<td>15.89</td>
<td>15.76</td>
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</tbody>
</table>

Sensory score: 7.0–9.0, very good; 4.0–6.9, good; 1.0–3.9, spoiled.
$L^*$, Brightness; $a^*$, red–green; $b^*$, yellow–blue.
MAP, modified-atmosphere packaging.
A study by Gerdes and Valdez noted that $L^*$ values of the red snapper decreased in all treatments (O$_2$–CO$_2$–N$_2$ mix, vacuum and air) during storage (2°C) and that $a^*$ values decreased for all samples by the seventh day. The color became dark (enzymatic reactions may have occurred) and eventually green. There was no difference in $b^*$ values between their treatments. They found that $\Delta E$ values were higher in the air-packaged samples compared with the MAP samples by the seventh day, but were lower on the 21st day of storage. $\Delta E$ is an indicator of overall color change and is defined as:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$  \hspace{1cm} (1)

whereby $\Delta L = L_{\text{color}, i} - L_{\text{color}, j}$; $\Delta a = a_{\text{color}, i} - a_{\text{color}, j}$; and $\Delta b = b_{\text{color}, i} - b_{\text{color}, j}$, where colors $i$ and $j$ are any two colors of interest.

**pH Properties**

The pH was 6.5 at the beginning of the experiment, but increased during storage (Table 2). Its level reached 7.2 for the control group, 6.8 for the A group, and 7.0 for the B group by the 14th day of storage. Differences between groups were not significant ($P > 0.05$) during storage. A similar study has shown that there were no clear effects by the packaging treatment on the pH of vegetable salad with herring.

**Moisture content**

The initial moisture content of fish salad was 70.1% (Table 2). In the control, MAP A, and MAP B groups, moisture content was found to be 71.8%, 70.1%, and 71.3%, respectively, by the 14th day of storage. There was no significant difference ($P > 0.05$) between the moisture content of groups during storage. The greatest increase was observed in the control group, which was 2.5%. The increased moisture content might be explained by the moisture having transferred from the vegetables. The moisture content of beef in air packages has been found to be higher than samples packaged with a N$_2$ : CO$_2$ gas mixture of 8 : 2.

**Gas properties**

Oxygen concentration decreased from 21% to 1.2% for control packages and from 5% to 2.1% for MAP group A (Table 2). In MAP group B packages, O$_2$ was not determined during storage. In the control packages, CO$_2$ concentration increased from 0.3% to 13.6% during storage but had decreased in the MAP groups. In a similar study, CO$_2$ concentration initially decreased and then remained almost constant in MAP vegetable salads with herring. The shelf life of catfish fillets stored in a modified atmosphere has been studied by Reddy et al., who reported that the headspace O$_2$ concentration of MAP decreased to 0% by the day of spoilage after an initial increase. Headspace O$_2$ concentration of the control group decreased from 20.9% to 0%, but CO$_2$ increased.

It has been reported that due to the oxygen consumption of microorganisms, O$_2$ concentration decreases during storage either in over-wrapped packages or in gas packages. Despite the fact that the lid material of the over-wrapped packages that was used in the experiment was quite permeable, CO$_2$ concentration still increased from 0% to 1% due to microbial activity. At the beginning of storage, CO$_2$ levels decreased in the gas packages and then remained relatively constant.

**Total volatile bases nitrogen properties**

The initial TVB-N value of fish salad was 11.5 mg/100 g of fish (Table 3). At the seventh day of storage,
the TVB-N-value of control samples was higher ($P \leq 0.05$) than that of the MAP groups. This value reached 25.54 mg/100 g of fish for the control group, 20.12 mg/100 g of fish for group A, and 22.31 mg/100 g of fish for group B by the 14th day of storage. A rejection limit of 25 mg/100 g of fish has been proposed for cold-stored rainbow trout. In the present study, because we had boiled the fish when preparing samples, the TVB-N-values were lower than that of the limit of acceptance at the day of spoilage.

Designating the acceptance limit for lean fish as 25 mg/100 g fish, the shelf life for gutted cod has been determined as 3 days, and as 4 days for air-packaged fillets stored at $0 \pm 1 \, ^\circ C$. Microbiological properties

Microbiological counts of MAP fish salads generally increased more slowly than those for the air-packaged control samples (Table 3). Initially, the average value of aerobes was $2.7 \log$ c.f.u./g. This value increased after seven days ($6.9 \log$ c.f.u./g for control, $5.8 \log$ c.f.u./g for MAP group A, and $3.8 \log$ c.f.u./g for MAP group B). If $10^5$ microorganisms per gram is considered to be the total viable count (TVC) limit of acceptance, after seven days the fish salads of the control group were considered to be spoiled, were still acceptable in MAP group A, and good in MAP group B. All samples were above $7.5 \log$ c.f.u./g at the 14th day of storage.

It has been demonstrated that cooking extends the lag phase of rockfish fillets packaged in 100% air, but that rapid microbial growth then occurs. However, no significant microbial growth was observed in the MAP rockfish fillets until the 19th day of storage. The shelf life of MAP tilapia has been studied previously, and it was found that spoilage generally occurred when bacterial counts reached or exceeded $7.5 \log$ c.f.u./g.

The numbers of both $S. \text{ aureus}$ and mould–yeast were $1.5 \log$ c.f.u./g at the first day of storage. $Staphylococcus \text{ aureus}$ was still $1.5 \log$ c.f.u./g in group A samples, had decreased to $0.5 \log$ c.f.u./g in group B samples, and had increased to $2.6 \log$ c.f.u./g in the control group after seven days of storage. If $5 \times 10^2$ c.f.u./g is considered to be the spoilage limit for $S. \text{ aureus}$, it is clear that the number in the control group was very close to that of spoilage. The amount of mould and yeast increased during storage; it increased to $8.1 \log$ c.f.u./g in the control group, to $7.3 \log$ c.f.u./g in the MAP A group, and to $5.6 \log$ c.f.u./g in the MAP B group samples. $Staphylococcus \text{ aureus}$ and coliform analyses were done to assess the hygienic handling of samples. The number of $S. \text{ aureus}$ present was not significantly different ($P > 0.05$) between the control and MAP group A samples, but was significantly lower in the MAP group B samples compared with the control group after 14 days of storage ($P \leq 0.05$). Similar results were determined for yeasts and moulds. Neither $Salmonella$ sp. nor anaerobic microorganisms were detected in fish salads during storage. Coliforms increased to $3.4 \log$ c.f.u./g in the control group and MAP A group, and increased to $2.7 \log$ c.f.u./g in the MAP B group samples. There was no significant difference ($P > 0.05$) between the control and group A samples, but group B samples were significantly better ($P \leq 0.05$) than the other groups by the last day of storage. Coliform bacteria are not commonly present in seafood when freshly caught. They are land-borne microorganisms and their presence is the result of non-hygienic practices on fishing boats and at markets.

The initial count of coliforms in catfish fillets has been determined as $3.2 \log$ c.f.u./g. In air packages, counts were found to have increased and either reached or exceeded $6.5 \log$ c.f.u./g by the day of spoilage, demonstrating that the fillets were not in a good condition at the beginning of storage. The initial quality of fish must be good for packaging to be effective.

Modified atmosphere packaging was effective in extending the shelf life of fish salads at $4 \, ^\circ C$. Gas packaging delayed any microbiological activity and sensory changes in the fish salads. The shelf life and quality of the MAP groups were similar,

<table>
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<th>Parameters</th>
<th>Control Day 0</th>
<th>MAP (A) Day 7</th>
<th>MAP (B) Day 7</th>
<th>Control Day 14</th>
<th>MAP (A) Day 14</th>
<th>MAP (B) Day 14</th>
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<tbody>
<tr>
<td>TVB-N (mg/100 g fish)</td>
<td>11.5</td>
<td>19.6</td>
<td>25.5</td>
<td>17.2</td>
<td>20.1</td>
<td>18.6</td>
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<td>3.8</td>
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<tr>
<td>$Staphylococcus \text{ aureus}$</td>
<td>1.5</td>
<td>2.6</td>
<td>4.3</td>
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<td>3.5</td>
<td>0.5</td>
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<td>1.5</td>
<td>5.5</td>
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<td>5.3</td>
<td>7.3</td>
<td>4.6</td>
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<td>3.4</td>
<td>3.0</td>
<td>3.4</td>
<td>1.4</td>
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MAP, modified-atmosphere packaging.
indicating that both the treatments used for preparing the MAP groups were successful in extending the shelf life of fish salads.

ACKNOWLEDGMENT

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