APPLICATION OF LOW DENSITY POLYETHYLENE FILMS:
2-THEIR EFFECT ON THE REFRIGERATED STORAGE
STABILITY OF VACUUM PackAGED COLD-SMOKEd
HERRING
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ABSTRACT

The present investigation was carried out to assess effect of feasibility of using
some various packaging materials, i.e. untreated & treated low density polyethylene
(LDPE) films, of 180 microns thickness, with potassium sorbate or calcium propionate
(at the concentrations of 2 and 4% w/w), and polyamide/polyethylene (PA/PE) film.
The study was focused on the effect of these packaging materials on the physico-
chemical (i.e. water holding capacity, total volatile bases nitrogen, trimethylamine
nitrogen, biogenic amines such as histamine, putrescine, cadaverine, tyramine,
tryptamine and B-phenylethylamine), thiobarbituric acid value and pH value),
microbiological quality (i.e. total aerobic counts and psychrophilic bacterial counts)
and sensory attributes (i.e. flavor, texture and overall acceptability) changes during
refrigerated storage (5±2°C) of vacuum packaged cold-smoked herring (Clupea
harangus).

Results indicated that among the used treatments, vacuum packaged cold-
smoked herring samples in LDPE films containing either potassium sorbate or calcium
propionate at the concentration of 4% were found to be superior in most aspects and
effective in extending their shelf-life (unaccepted after 12 wk). Following it, those
samples of cold-smoked herring packaged in LDPE films with 2% potassium sorbate
or calcium propionate which were unacceptable after 10 wk). On the other hand,
samples of vacuum packaged in LDPE film and PA/PE film demonstrated major
changes in most quality attributes that were obviously observed, being unacceptable
after 8 wk of storage at 5±2°C.

Finally, using the antimicrobial low density polyethylene films containing 2% and
4% potassium sorbate or calcium propionate could be recommended for
packaging vacuum cold-smoked herring. Such packaging would only be affected for
keeping the quality attributes of the product but also could extended its shelf-life.

Keywords: Smoked herring, antimicrobial packaging film, vacuum packaging,
kashmir sorbate, calcium propionate, quality attributes.

INTRODUCTION

Polyethylene bags are commonly used as packaging material due to
their low cost and convenience (Ho, et al., 1995).

Recently, the idea of incorporating antimicrobial agents into polymeric
packaging films has been developed for improving the qualities of packaged
foods. Therefore, the films have a potential for improving microbial stability of
food by acting on the food surface which is in contact with them. These films
can work as a food preservative media, maintaining high local concentration
of antimicrobial agents, while reducing their total amount in the food
(Hotchkiss, 1995).
In packaging science, migration is the process by which residues from polymerization or additives present in the polymer to improve processability diffuse through the polymer matrix to the inner package surface (in contact with food) where they are solved by food and dissolved (Garde, et al., 1998). The migration depended on the structure of the polymer, density of the plastic, concentration of small molecule in the plastic, contact time of the plastic with the food, structure of the food, temperature and other physicochemical properties (Figge, 1980).

Since microbial growth in solid and semi-solid foods such as meat and meat products appeared to occur primarily at the surface, attempts have been made to delay spoilage by the use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods was found to have limited benefits because the active substances were neutralized on contact or diffused rapidly into the bulk food, away from the surface (Siragusa and Dickson, 1992). To overcome this problem, some attempts made to develop active packages, in which antimicrobial agents would be incorporated and slowly released at the food surface, where they remain at high concentrations for extended period of time (Kester and Fennema, 1986; Hatchkiss, 1995 and Gennadios, et al., 1997).

The application of antimicrobial films to control and inhibit the growth of bacterial spoilage, included the use of edible corn starch film containing potassium sorbate and lactic acid (Baron, 1993); chitosan films impregnated with acetic or propionic acid (Ouattara, et al., 2000) and antimicrobial edible films with sorbic or p-aminobenzoic acid (Cagri, et al., 2002).

The shelf-life of ready to eat fish products i.e. smoked fish, is a function of several factors, including effect of smoke constituents, loss in moisture, salt concentration in presmoking salt treatment, degree of smoking temperature, permeability of various packaging materials, kinds of packaging and storage temperature (Hildebrandt and Erol, 1988).

The present study was carried out to investigate the feasibility of using antimicrobial low density polyethylene packaging films for extending the shelf-life of vacuum packaged cold-smoked herring (Clupea harengus) stored at 5±2°C.

**MATERIALS AND METHODS**

**Fabrication of antimicrobial low density polyethylene (LDPE) films:**

Fine powders of potassium sorbate or calcium propionate (60-90 microns) at the concentrations of 2 and 4% (w/w) were mixed with low density polyethylene powder (Density = 0.915 g/cm³, Mw=35000, melt index =0.22 and Tm=115°C, Polychemical Corporation R 41661 NOVATAC LES42 H, Japan). The mixtures were then extruded by the blown film extrusion process in a single screw extruder in Zarka Plast Co., Egypt, which was 40 cm long and had a diameter 5 cm at a screw speed of 50 rpm and the temperature was maintained in the range of 95 to 120°C during the extrusion process of the films. The film produced by this system was 10 to 15 cm wide and had a thickness of 180 microns.
Processing of smoked fish:
Frozen herring (Clupea harengus) was obtained and cold-smoked in El-Negma Company for Fish Processing, Cairo, Egypt, as follows: The fishes were thawed at room temperature for 12 hr, salted by mixing fish with dry sodium chloride at a ratio of 5:1 (w/w) for 6 days, rinsed by tap water to remove the excess salt, the salted fish was hanged upside down on racks and allowed to drainage for 2 days, then they were cold-smoked in smokehouse at 30-35°C for 4 days, in which smoke was produced from a sawdust and wood shavings. After smoking process, the fishes were cooled to room temperature, divided into six portions, then individually vacuum packaged in differed packaging materials. One portion was packaged in PA/PE film. Four portions were packaged in antimicrobial LDPE films containing 2% and 4% of either potassium sorbate or calcium propionate. While, the sixth portion was packaged in untreated LDPE film as control film. All the samples were stored in the refrigerator at 5±2°C for the possible storage periods (8-12 weeks). The smoked fish was immediately analyzed after the smoking process and periodically every two weeks of storage for assessing the physical, chemical, microbial and sensory attributes.

Analytical methods:
The moisture, protein, fat, ash and sodium chloride contents were determined according to methods of A.O.A.C (1995).
Total volatile bases nitrogen (TVBN) and trimethylamine nitrogen (TMA-N) were determined according to the method of Malle and Tao (1987).
Biogenic amines determination: Biogenic amines analysis for histamine, cadaverine, putrescine, tyramine, tryptamine, and B-phenylethylamine, was carried out by high-performance liquid chromatography (HPLC). Preparation of biogenic amines standard solutions and derivatization of sample extracts and standards were carried out as described by Mietz and Karmas (1978) and Maijala, et al. (1993). Waters HPLC system equipped 600 delivery system. HPLC column: Reverse phase Nova-Pak C18 Waters column 3.9x15 mm, 60Å, 4μm. The detection was performed using UV detector (Waters 496) at 254 nm wavelength. The used program was 75% solvent B [0.2 N acetic acid: acetonitrile: methanol (1:9:9)] in solvent A [Acetonitrile: 0.02 N acetic acid (1:9)] to 100% solvent B, using linear program of 25 min period and 1ml/min constant solvent flow rate. Sample volume of 10 μl was injected. Data was integrated and recorded using a Millennium Chromatography Manager Software 2001 (Waters, Milford MA 01767). Concentrations of amines (mg/kg fish flesh) were calculated from peaks area of pure amines and examined samples which obtained from the chart.
Vyncke’s method (1970) was performed for determination of thiobarbituric acid (TBA) value and calculated as mg malonaldehyde/kg fish flesh.
The pH value of fish was determined in the slurry according to the method described by Scott, et al. (1988) using Orion pH-meter (model SA 720, USA).
El-Akel, A. T.

Determination of total amounts of potassium sorbate or calcium propionate released from LDPE films:

The releasing amount of potassium sorbate from LDPE films was determined according to the A.O.A.C. (1995) method. Triplicate samples of 10 cm diameter and 1.5 cm thickness was used to assess sorbic acid amount in the sample by means of ultraviolet spectrophotometry after extracting with ethyl ether in an acid medium. A double-beam UV-visible spectrophotometer model UV-4050 (LKB Biochrom. Ultraspec II, Anoutstanding British product) was used, and the absorbance was determined at 250 nm. Releasing amount of calcium propionate from LDPE films into herring smoked was carried out as described by Ouattara, et al. (2000), using high-performance liquid chromatography HP 1090 Multisolvent Delivery System (Hewlet PACKARD). Peak separation was achieved through ODS dp 5µL (4.6 x 25 mm) Ultrasphere stainless steel column, using a 0.005 N sulfuric acid solution as the mobile phase, at a flow rate of 1m/min. Detection was done at 210 nm, on a DAD wavelength detector and Spectra Focus optical scanning detector. Potassium sorbate and calcium propionate concentrations were determined throughout storage and were expressed as ppm sorbic acid and propionic acid, respectively, based on sample weight.

Water holding capacity (WHC) of fish samples was measured according to the method described by Wierbicki and Detherage (1958). The W.H.C (cm³/0.3 g) was calculated by subtracting the area of internal zone from that of the outer one.

Microbiological analysis:

Ten gram sample of smoked herring was blended with 90 ml sterile peptone buffered solution (0.1% w/v) using a high speed blender for 2 min and decimal serial dilutions were then prepared from the homogenate for counting the total aerobic & psychrophilic bacteria counts, which were determined on tryptone glucose yeast agar (5666) and the inoculated plates were incubated at 30°C for 2 days and 5°C for 5 days, respectively, according to the methods described by Difco Manual (1984).

Sensory evaluations:

A panel test was done by ten staff members from Food Technology Dept., Fac. of Agric., Cairo Univ., to evaluate the organoleptic qualities of the smoked herring samples, i.e. flavor and overall acceptability. A nine point hedonic scale ranging from extremely like (10) to extremely dislike was used for quality scoring according to the method of Teeny and Miyauchi (1972).

Statistical analysis:

The obtained data were statistically analyzed by analysis of variance and least significant difference test (L.S.D) according to Snedecor and Cochran (1980).
RESULTS AND DISCUSSION

Chemical composition, quality attributes and microbial aspects of the freshly cold-smoked herring:

The chemical composition, quality attributes and microbial aspects of the freshly cold-smoked herring were determined and illustrated in (Tables 1 & 2).

Results indicated that the moisture content of cold-smoked herring represents 51.91% of total muscle weight and their contents of protein, crude fat and ash (On fresh weight basis) were 30.39%, 8.15% and 9.55%, respectively.

Table (1): Chemical composition of the freshly cold-smoked herring.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>On fresh wt. Basis (g/100 g fish flesh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>51.91</td>
</tr>
<tr>
<td>Crude protein</td>
<td>30.39</td>
</tr>
<tr>
<td>Crude fat</td>
<td>8.18</td>
</tr>
<tr>
<td>Ash</td>
<td>9.52</td>
</tr>
</tbody>
</table>

Furthermore, the quality attributes in Table (2) showed that the total volatile bases nitrogen (TVBN) and trimethylamine nitrogen (TMA-N) were 15.04 and 1.26 mg/100g fish flesh, respectively. The thiobarbituric acid (TBA) value was 0.981 mg malonaldehyde/kg fish flesh. These values for the freshly cold-smoked herring were considered low. In this respect, Cantoni, et al., (1993) reported that, the maximum acceptable levels of TMA-N and TVBN in smoked fish were 8-10 and 30 mg/100 g fish flesh, respectively. According to the Egyptian Standards (1996), smoked fish should not contain more than 10 mg/100g fish flesh of TMA-N, while, TBA value should not be more than 1 mg malonaldehyde/Kg fish flesh as recommended by Chang, et al. (1961).

Data in (Table 2) also indicated that, the total aerobic count in smoked herring was $3.1 \times 10^3$ CFU/g fish flesh, being less than the limit ($10^5$ cell/g) of the Egyptian Standards (1996) and the recommended microbiological limits in fish ($10^7$ CFU/g) (ICMSF, 1986). From the view point of microbial load, these findings indicated to the high quality of smoked herring.

In addition, data in (Table 2) demonstrated that the freshly cold-smoked herring contained low amounts of cadaverine and putrescine being 0.921 and 14.173 mg/kg fish flesh, respectively. However, the others biogenic amines i.e. tryptamine, B-phenylethylamine, histamine and tyramine, were not detectable.

Results in (Table 2) show that, the freshly smoked herring had low water holding capacity (5.31 cm³).

From the above results, it could be concluded that, freshly smoked herring had high degree of quality attributes from both chemical and microbiological points of view according to the studies of other workers (Mackie, et al., 1997; Fletcher, et al., 1998, Gingerich, et al., 1999 and Ozogul, et al., 2002).
Table 2: Quality attributes of freshly cold-smoked herring.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>On fresh wt. Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volatile bases nitrogen (TVBN) (mg/100g)</td>
<td>15.04C</td>
</tr>
<tr>
<td>Trimethylamine nitrogen (TMA-N) (mg/100g)</td>
<td>1.260</td>
</tr>
<tr>
<td>Thiobarbituric acid (TBA) value (mg/kg)</td>
<td>0.981</td>
</tr>
<tr>
<td>pH value</td>
<td>6.065</td>
</tr>
<tr>
<td>Salt content (g/100g)</td>
<td>7.750</td>
</tr>
<tr>
<td>Biogenic amines (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>1. Tryptamine</td>
<td>N.D.</td>
</tr>
<tr>
<td>2. Putrescine</td>
<td>14.173</td>
</tr>
<tr>
<td>3. B-phenylethylamine</td>
<td>N.D.</td>
</tr>
<tr>
<td>4. Cadaverine</td>
<td>0.921</td>
</tr>
<tr>
<td>5. Histamine</td>
<td>N.D.</td>
</tr>
<tr>
<td>6. Tyramine</td>
<td>N.D.</td>
</tr>
<tr>
<td>Water holding capacity (cm²)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Microbial aspects (CFU/g)</td>
<td></td>
</tr>
<tr>
<td>1. Total aerobic count</td>
<td>3.1x10⁴</td>
</tr>
<tr>
<td>2. Psychrophilic count</td>
<td>1.3x10⁴</td>
</tr>
</tbody>
</table>

N.D. = Not detectable.

Effect of antimicrobial LDPE films on the quality attributes of vacuum packaged cold-smoked herring during storage at 5±2°C.

The changes on the quality attributes of vacuum packaged cold-smoked herring during storage at 5±2°C as affected by various packaging materials were carried out periodically every two weeks during storage period at 5±2°C.

Changes in moisture content:

The obtained results (Table 3) indicated that slight differences were observed between the moisture contents of samples packaged in LDPE films treated with and without potassium sorbate or calcium propionate (at concentrations of 2% and 4% w/v). The moisture contents of these samples were higher than that of those packaged in PA/PE films. Moreover, the moisture contents for the all samples of vacuum packaged cold-smoked herring tended to gradually decrease during refrigeration storage for 14 weeks at 5±2°C. This decrease in moisture content during storage of smoked herring could be ascribed by the evaporation of water inside the polyethylene bags as well as a continuous hydrolysis of protein and consequently the decrease in water-holding-capacity which led to the loss of moisture content (El-Kholy, 1994).
**Table (3): Moisture contents (%) of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5±2°C.**

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>PA/PE** (control film)</th>
<th>LDPE Film* with potassium sorbate 2%</th>
<th>LDPE films impregnated with potassium sorbate 4%</th>
<th>LDPE films impregnated with calcium propionate 2%</th>
<th>LDPE films impregnated with calcium propionate 4%</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>51.91</td>
<td>51.91</td>
<td>51.91</td>
<td>51.91</td>
<td>51.91</td>
</tr>
<tr>
<td>2</td>
<td>50.32</td>
<td>50.92</td>
<td>50.84</td>
<td>50.41</td>
<td>50.62</td>
</tr>
<tr>
<td>4</td>
<td>49.52</td>
<td>50.56</td>
<td>50.23</td>
<td>49.89</td>
<td>49.89</td>
</tr>
<tr>
<td>6</td>
<td>47.88</td>
<td>49.51</td>
<td>48.82</td>
<td>48.64</td>
<td>48.42</td>
</tr>
<tr>
<td>8</td>
<td>45.21</td>
<td>48.98</td>
<td>48.15</td>
<td>47.91</td>
<td>47.70</td>
</tr>
<tr>
<td>10</td>
<td>43.41</td>
<td>48.05</td>
<td>47.21</td>
<td>46.81</td>
<td>46.86</td>
</tr>
<tr>
<td>12</td>
<td>41.90</td>
<td>47.41</td>
<td>46.78</td>
<td>46.39</td>
<td>46.23</td>
</tr>
<tr>
<td>14</td>
<td>40.51</td>
<td>47.15</td>
<td>46.21</td>
<td>45.25</td>
<td>45.48</td>
</tr>
</tbody>
</table>

* LDPE film: Untreated low density polyethylene film with potassium sorbate or calcium propionate.
** PA/PE: Polyamide / polyethylene film.

It is obvious that, the changes in moisture contents of vacuum packaged cold-smoked herring in untreated and treated LDPE films with either potassium sorbate or calcium propionate (at concentrations of 2% and 4% w/w) after different periods of refrigeration storage were lower than those for the samples packaged in PA/PE films. Furthermore, smoked herring packaged in LDPE films containing either 4% potassium sorbate or calcium propionate showed generally lower moisture contents than those in LDPE films with 2% potassium sorbate or calcium propionate. This may be due to the different water-vapor permeability. Since, LDPE containing 4% potassium sorbate or calcium propionate exhibited higher water-vapor permeability values (0.3 g/m²/48 hrs at 90% RH/28°C, respectively) than untreated LDPE film (0.5 g/m²/48 hrs at 90% RH/28°C) which was previously found (El-Akel, 2003). Thus, these films (antimicrobial films) could be considered as suitable packaging materials which maintain the moisture content at the required (recommended) level. Similar results were also found by other workers (Jenkins and Harrington, 1991 and Hirsch, 1991) who reported that, polyethylene films provide good barrier to moisture, while, polyamide/polyethylene films provide poor barrier to moisture and allow changes in moisture content of the packaged products.

**Changes in total volatile bases nitrogen (TVBN) and trimethylamine nitrogen (TMA-N):**

Total volatile bases nitrogen and trimethylamine nitrogen are frequently measured for detecting the spoilage of fish and its products (Gram and Huss, 1996). Moreover, TVBN includes TMA-N and other compounds such as ammonia and TMA results from the reduction of trimethylamine oxide by some aerobic and anaerobic microorganisms placed in low-oxygen conditions (Lerol and Joffraud, 2000).
Fig. (1): Total volatile bases nitrogen (mg/100g herring flesh) of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5±2°C.

Fig. (2): Trimethylamine nitrogen (mg/100g herring flesh) of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5±2°C.
Data illustrated in Figs. (1&2) show the changes occurred in TVBN and TMA-N of vacuum packaged cold-smoked herring in various protective wraps. It could be noticed that, TVBN and TMA-N values of either LDPE or PA/PE-packaged cold-smoked herring were progressively increased as the period of cold storage was increased. After 10 weeks of cold storage these values reached 35.86 and 33.81 mg/100 g fish flesh (On fresh wt. basis), respectively, while TMA-N values were 11.76 and 10.92 mg/100 g fish flesh (On fresh wt. basis), respectively. These results showed unacceptable occurred changes which leading to refuse the smoked herring according to the recommended limits of TVBN and TMA-N as reported and suggested for smoked fish by Cantoni, et al. (1993) and Egyptian Standards (1996) (≤ 30 and 8-10 mg/100g fish flesh, respectively).

On the other hand, it could be also observed from the same Figs. that, at any time of cold storage at 5±2°C, values of TVBN and TMA-N of cold-smoked herring vacuum packaged in LDPE films impregnated with potassium sorbate or calcium propionate (at concentrations of 2 and 4%) were lower than those vacuum samples in the other packaging materials i.e. LDPE and PA/PE films. In addition, the results (Figs 1&2) also indicate that, TVBN and TMA-N values of smoked herring vacuum packaged in LDPE films containing potassium sorbate or calcium propionate (4%) were lower than those of vacuum packaged in LDPE films with the same materials at the concentration of 2%. Values of TMA-N obtained for vacuum packaged cold smoked herring in LDPE films impregnated with potassium sorbate or calcium propionate (at concentrations of 4 and 2%) fluctuated from 8.61 to 10.08 and 8.61 to 9.66 mg/100g fish flesh, after 12 and 10 weeks of cold storage, respectively, being within the acceptable limits as reported by Cantoni, et al. (1993). This might be due to that the releasing of such preservatives from the antimicrobial LDPE films to control the growth of spoilage bacteria were increased by increasing their concentrations in the films. In this concern, Baron, (1993) found that, the application of edible corn starch film containing potassium sorbate and lactic acid inhibited the growth S. typhimurium and E. coli 0157:H7 on poultry. Similarly, antimicrobial chitosan films containing acetic or propionic acid inhibited growth of Enterobacteriaceae and Serratia liquefaciens on bologna (Ouattara, et al., 2000). Moreover, a decline numbers of E. coli 0157:H7 on bologna slices was observed after 21 days at 4°C using antimicrobial films containing sorbic acid or p-aminobenzoic acid (at 0.5 to 1.0%) (Cagri, et al., 2002). These results emphasize the potential for adding antimicrobial compounds such as potassium sorbate or calcium propionate to packaging materials.

The above mentioned results coincide with those found in this study concerning the residual contents of either potassium sorbate or calcium-propionate. As the residual contents of potassium sorbate and calcium propionate in cold-smoked herring packaged in LDPE films impregnated with 2% potassium sorbate or calcium propionate were 308 and 569 ppm (as sorbic acid and propionic acid), respectively, after 10 weeks storage. However, the residual contents reached 569 ppm (as sorbic acid) and 1092 ppm (as propionic acid) in cold-smoked herring packaged in LDPE film with 4% potassium sorbate or calcium propionate after 12 weeks storage at 5±2°C.
These results indicated that, the amount of potassium sorbate or calcium propionate in LDPE films migrated into smoked herring were increased with increasing their concentrations. It could be also mentioned that, these values were lower than the Acceptable Daily Intakes. According to FAO/WHO (1974) sorbic acid intake should not exceed 1500 mg sorbic acid for a 60 kg person. Moreover, potassium sorbate is effective in most foods at the concentration range of 0.05-0.3% by weight (Sofos, et al., 1980). According to Egyptian Standards for Food Additives Permitted (1993), the maximum permissible limit for propionic acid is 3000 ppm in foods.

Changes in biogenic amines content:

Biogenic amines are produced by spoilage bacteria (i.e. mesophilic flora, Enterobacteriaceae and coliforms) towards the end of shelf-life of a fish. Their levels are considered as indices of spoilage rather than freshness. Furthermore, determination of biogenic amines is important not only from the point of view of their toxicity, but also can be used as indicator for the decomposition of fish (Mietz and Karmas, 1978 and Mackie, et al., 1997).

Biogenic amines were determined in the samples packaged in LDPE films containing 4% potassium sorbate or calcium propionate, having the maximum possible shelf-life and compared with PA/PE and LDPE control films.

The concentrations of biogenic amines present in vacuum packaged cold-smoked herring samples after refrigerated storage for 10 weeks in PA/PE & untreated LDPE films and 12 weeks in treated LDPE films with potassium sorbate or calcium propionate (at concentrations of 4% w/w) are shown in Table 4. After the above mentioned storage periods, smoked herring samples were unacceptable according to the limiting levels of TMA-N and TVBN which are 8-10 and 30 mg/100g fish flesh, respectively.

Table (4): Biogenic amines (mg/kg fish flesh) of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5+2°C.

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>Packaging materials</th>
<th>Biogenic amines (mg/kg)</th>
<th>Tryptamine</th>
<th>B-phenylethylamine</th>
<th>Putrescine</th>
<th>Cadaverine</th>
<th>Histamine</th>
<th>Tyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>LDPE film*</td>
<td>280.730</td>
<td>106.41</td>
<td>5.348</td>
<td>4.272</td>
<td>3.292</td>
<td>1.089</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>PAPE**</td>
<td>187.155</td>
<td>116.235</td>
<td>5.434</td>
<td>N.D</td>
<td>N.D</td>
<td>0.559</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>LDPE film with 4% potassium sorbate</td>
<td>N.D</td>
<td>5.103</td>
<td>0.217</td>
<td>4.476</td>
<td>0.108</td>
<td>1.118</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>LDPE film with 4% calcium propionate</td>
<td>N.D</td>
<td>12.117</td>
<td>1.425</td>
<td>3.002</td>
<td>0.291</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* LDPE film: Untreated low density polyethylene film with potassium sorbate or calcium propionate.
** PAPE: Polyamide / polyethylene film.
N.D.: Not detected.

It could be noticed that, the biogenic amines contents (i.e. histamine, putrescine, cadaverine, tyramine, tryptamine and B-phenylethylamine) of the samples packaged in LDPE films impregnated with potassium sorbate or calcium propionate (at the concentration of 4%) were the lowest values even.
after 14 weeks of storage at 5±2°C as compared to the other samples after storage for only 10 weeks at the same temperature. This could be attributed to the antimicrobial effect of potassium sorbate or calcium propionate which released from antimicrobial films to smoked herring during cold storage as previously reported (Baron, 1993; Ouattara, et al. 2000 and Cagri, et al. 2002).

In addition, the highest concentration of biogenic amines was shown in vacuum packaged cold-smoked herring sample kept in LDPE film, followed by the corresponding sample stored in PA/PE films after cold storage for 10 weeks.

Data in (Table 4) shows that, the histamine contents in these packaged smoked herring in various packaging materials after 10 and 14 weeks of cold storage at 5±2°C varied between 0.108 to 3.292 mg/kg herring flesh, when the samples were organoleptically rejected. These values were less than the legal limit and advisory level (5 mg/100g fish flesh) which was established by Food and Drug Administration (FDA, 1996) and would not be expected to cause symptoms of scombrototoxin poisoning (Zotos, et al., 2001). Thus, the level of histamine in vacuum packaged cold-smoked herring could not be used to measure their decomposition or serve as an indicator for their quality.

Similarly, results in the same Table revealed that, the concentrations of biogenic amines such as putrescine, cadaverine and tyramine were also low in vacuum packaged smoked herring samples in all the used packaging materials after the possible storage period.

On the other hand, both LDPE and PA/PE samples contained comparatively higher values of tryptamine and B-phenylethylamine compared with those of vacuum packaged cold-smoked herring in LDPE films impregnated with 4% potassium sorbate or calcium propionate. These results indicated that, ingestion of these samples may cause some health problems since tryptamine and phenylethylamine act as potentiators of histamine toxicity and raise blood pressure (Koehler and Eltenmiller, 1978 and Taylor, et al., 1984).

Changes in thiobarbituric acid (TBA) value:

Thiobarbituric acid value is used as an index for measuring oxidative rancidity (malonaldehyde formation), which might take place in cold-smoked herring during the refrigerated storage.

Data presented in Fig. 3 showed the changes in the TBA values of vacuum packaged cold-smoked herring in untreated and treated LDPE films with potassium sorbate or calcium propionate (at the concentrations of 2% and 4%) as well as in PA/PE film throughout different storage periods at 5±2°C.

The obtained results indicated that, TBA values of smoked herring packaged in LDPE film were higher than those packaged in the other used protective films during refrigerated storage at 5±2°C up to 8 weeks. A marked increase in their TBA value (1.22 mg/kg herring flesh) was only obtained after 10 weeks of cold storage at 5±2°C. In this respect, Chang, et al. (1961) reported that, off flavor attribute in meat tissues could be noted when T.B.A value reached 1.0 mg malonaldehyde/kg. This may be due to the poor O₂
barrier properties of LDPE film which cause fat oxidation (El-Sayed, 1998). Moreover, vacuum packaged cold-smoked herring in PA/PE films showed the lowest TBA values at any time of cold storage as compared with those packaged in the other used packaging materials. This may be attributed to the better O₂ barrier properties of PA/PE film compared to LDPE film (El-Gazar, 1997). Regardless, vacuum packaged cold-smoked herring in LDPE films containing 2 and 4% potassium sorbate or calcium propionate had higher TBA values than those samples in PA/PE, they still contain acceptable TBA values that not exceeding 1.0 mg/kg fish flesh as reported by Chang, et al. (1961) and Zhao and Sebranek (1996), after storage up to 10 and 12 weeks, respectively. This results may be due to the low O₂ permeability values for LDPE films impregnated with 4% potassium sorbate or calcium propionate. These values were 707 cc/m²/24 hr at 22-25°C and 965 cc/m²/24 hr at 22-25°C, respectively (compared with untreated LDPE that was 1063 cc/m²/24 hr at 22-25°C which were found in the previous work (El-Akel, 2003). Therefore, the used antimicrobial LDPE films could be more effective in reducing TBA values of vacuum packaged cold-smoked herring during refrigerated storage compared with untreated LDPE. This effect could be attributed to the release of potassium sorbate or calcium propionate from the antimicrobial LDPE films into smoked herring to control the growth of lipolytic activities of pseudomonas and Gram-negative bacteria which could influence the oxidation and rancidity of fats. Similar results were reported by the other works using different packaging materials (Huang and Lin, 1995; Ouattara, et al., 2000 and Cagri, et al., 2002).

Thus, antimicrobial LDPE films with potassium sorbate or calcium propionate would be more effective in reducing TBA values than LDPE film and consequently prolonging storage life of smoked herring.

Changes in the pH value:

Data in Fig. 4 represents the changes in the pH values of vacuum packaged cold-smoked herring in PA/PE, untreated and treated LDPE films during cold storage at 5±2°C for 14 weeks.

The highest pH values were recorded for samples packaged in LDPE film, followed by the corresponding samples in PA/PE film. However, those packaged in antimicrobial LDPE films had the lowest values at any stage of the storage time (14 weeks). Therefore, if seemed that, antimicrobial LDPE films protected the vacuum packaged cold-smoked herring from some bacterial changes, leading to increase of pH value as previously reported by other researchers using other antimicrobial films (Baron, 1993; Ouattara, et al., 2002 and Cagri, et al., 2002). Moreover, the increase of pH values of cold-smoked herring were almost parallel to the increased of its TVBN values (Fig. 1). These results support the findings of El-Kholy, (1994) and Gouda, (2002) who found that, the pH value increased during storage, due to the action of bacterial breakdown and formation of some alkaline substances which lead to pH rise.
Fig. (3): Thiobarbituric acid values (mg malonaldehyde/kg herring flesh) of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5±2°C.

Fig. (4): pH values of vacuum packaged cold-smoked herring in various packaging materials during refrigerated storage at 5±2°C.
Changes in water holding capacity (WHC):

Water holding capacity is one of the more important physical characteristics of meat and fish and mainly affect their textures as well as its relation to the proteins quality and quantity. It should be mentioned that in the area of the formed zone is small then the WHC of the tested samples is high and vice versa.

Data in Fig. 5 showed the changes in WHC of vacuum packaged cold-smoked herring in PA/PE, untreated & treated LDPE films during cold storage at 5±2°C for 14 weeks. The highest decrease of WHC were recorded for samples packaged in LDPE film, followed by the corresponding samples in PA/PE film. However, the lowest values of WHC were observed for those packaged in antimicrobial LDPE films at any stages of storage time (14 weeks). Moreover, the decrease of WHC of cold-smoked herring were almost parallel to the increase of its TMA-N and TVBN values (Figs. 1&2). Therefore, antimicrobial LDPE films retarded the decline of WHC of smoked herring during storage and might be possibly due to the release of potassium sorbate or calcium propionate from LDPE films into smoked herring to control the growth of some bacteria on the fish compounds as found by others workers (Huang and Lin, 1995; Ouattara, et al., 2000 and Cagri, et al., 2002).

Microbial counts of vacuum packaged cold-smoked herring during refrigerated storage:

Data in Table (5) showed total aerobic plate counts of vacuum packaged cold-smoked herring in various packaging materials throughout the storage period at 5±2°C.

Table (5): Microbial counts of vacuum packaged cold-smoked herring during refrigerated storage at 5±2°C.

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>PA/PE*</th>
<th>LDPE** (Control film)</th>
<th>LDPE films impregnated with potassium sorbate</th>
<th>LDPE films impregnated with calcium propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>3.1×10⁷</td>
<td>1.3×10⁷</td>
<td>3.1×10⁷</td>
<td>1.3×10⁷</td>
</tr>
<tr>
<td>2</td>
<td>1.3×10⁶</td>
<td>4.1×10⁶</td>
<td>7.1×10⁷</td>
<td>2.7×10⁷</td>
</tr>
<tr>
<td>4</td>
<td>8.1×10⁵</td>
<td>2.7×10⁷</td>
<td>6.9×10⁷</td>
<td>2.6×10⁷</td>
</tr>
<tr>
<td>6</td>
<td>4.9×10⁵</td>
<td>6.4×10⁷</td>
<td>7.3×10⁷</td>
<td>2.4×10⁷</td>
</tr>
<tr>
<td>8</td>
<td>4.9×10⁵</td>
<td>6.4×10⁷</td>
<td>7.3×10⁷</td>
<td>2.4×10⁷</td>
</tr>
<tr>
<td>10</td>
<td>5.9×10⁵</td>
<td>6.6×10⁷</td>
<td>5.8×10⁷</td>
<td>3.0×10⁷</td>
</tr>
<tr>
<td>12</td>
<td>5.9×10⁵</td>
<td>6.6×10⁷</td>
<td>5.8×10⁷</td>
<td>3.0×10⁷</td>
</tr>
<tr>
<td>14</td>
<td>5.9×10⁵</td>
<td>6.6×10⁷</td>
<td>5.8×10⁷</td>
<td>3.0×10⁷</td>
</tr>
</tbody>
</table>

* PA/PE: Polyamide / polyethylene film.
**LDPE: Untreated low density polyethylene film with potassium sorbate or calcium propionate.
A: Total aerobic counts. B: Psychrophilic bacteria counts.

Data demonstrated that, the total aerobic plate counts of vacuum packaged samples in PA/PE and LDPE films successively increased during storage reaching 6.9×10⁷ and 7.5×10⁷ CFU/g fish flesh after storage for 10 weeks, respectively. Similarly, Gouda (2002) found that, the aerobic plate counts level reached over 10⁷ CFU/g after only 4 weeks at 5°C.
Fig. (5): Changes in water holding capacity (WHC) (cm$^2$) of vacuum packaged cold-smoked herring during storage at $5 \pm 2^\circ$C.
On the contrary, the lowest total aerobic plate counts were detected in vacuum packaged cold-smoked herring in LDPE films containing 4% potassium sorbate or calcium propionate, followed by LDPE films containing 2% potassium sorbate or calcium propionate. After 12 and 14 weeks, respectively, their total aerobic plate counts accounted over $10^7$ CFU/g (unacceptable limit recommended by ICMSF, 1985). These results could be ascribed by the release of potassium sorbate or calcium propionate from the antimicrobial LDPE films and subsequently reaching or inhibiting the microbial growth on smoked herring. Their inhibitory effectiveness was more pronounced as the concentrations of the used antimicrobial agents were increased in the LDPE films. Data in the same table demonstrated that, the counts of psychrotrophic bacteria showed a similar behaviour during the storage as the total aerobic plate counts of smoked herring. These results are in consistency with those reported by previous researchers (Baron, 1993, Ouattara, et al., 2000, Cagri, et al., 2002 and Janes, et al., 2002) using antimicrobial packaging films with organic acids for the preservation of meat products.

Finally, it could be concluded that the spoilage of vacuum packaged cold-smoked herring was associated with high numbers of microorganisms, since, the development of TMA-N was related to growth and activity of microorganisms.

Organoleptic evaluation:

Table (6) represented the mean values of sensory attributes (flavor, texture and overall acceptability) of vacuum packaged cold-smoked herring during refrigerated storage at 5±2°C.

All the acceptability scores for flavor, texture and overall acceptability of LDPE and PA/PE vacuum packaged cold-smoked herring significantly decreased after storage for 8 weeks. Therefore, samples were rejected by the panelists.

On the other hand, vacuum packaged cold-smoked herring in LDPE films containing 2% and 4% potassium sorbate or calcium propionate, remained acceptable after cold storage at 5±2°C up to 10 and 12 weeks, respectively. Afterwords, the acceptability scores tended to significantly decrease and these samples were completely rejected after 12 and 14 weeks, respectively.

It is worthy to mention that, among the different samples, vacuum packaged cold-smoked herring in antimicrobial LDPE films either containing potassium sorbate or calcium propionate at the concentration of 4% had the highest platability scores. Such treatments have potentially proven for using the antimicrobial films for keeping the organoleptic qualities during cold storage at 5±2°C. These findings coincide with those obtained for both chemical and microbial evaluations as previously discussed.

Thus, using antimicrobial LDPE films impregnated either with 2% and 4% potassium sorbate or calcium propionate could be recommended for packaging the smoked fish to enhance product safety and extend their shelf-life.
Table (6): Sensory attributes of vacuum packaged cold-smoked herring in various packaging materials during storage at 5±2°C.

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Storage Period (weeks)</th>
<th>PA/PE**</th>
<th>LDPE Film***</th>
<th>LDPE films containing potassium sorbate</th>
<th>LDPE films containing calcium propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.9</td>
<td>8.5†</td>
<td>8.5†</td>
<td>8.9†</td>
<td>8.9†</td>
</tr>
<tr>
<td>2</td>
<td>8.7</td>
<td>8.7‡</td>
<td>8.8§</td>
<td>8.8§</td>
<td>8.8§</td>
</tr>
<tr>
<td>4</td>
<td>8.4</td>
<td>8.4</td>
<td>8.6†</td>
<td>8.6†</td>
<td>8.6†</td>
</tr>
<tr>
<td>6</td>
<td>8.2</td>
<td>8.1</td>
<td>8.4‡</td>
<td>8.6†</td>
<td>8.6‡</td>
</tr>
<tr>
<td>8</td>
<td>8.1</td>
<td>7.7‡</td>
<td>8.2</td>
<td>8.4‡</td>
<td>8.1‡</td>
</tr>
<tr>
<td>10</td>
<td>7.3</td>
<td>7.1†</td>
<td>7.7‡</td>
<td>8.3‡</td>
<td>7.7‡</td>
</tr>
<tr>
<td>12</td>
<td>8.5</td>
<td>7.8‡</td>
<td>7.2‡</td>
<td>8.4‡</td>
<td>7.4‡</td>
</tr>
<tr>
<td>14</td>
<td>8.8</td>
<td>8.3§</td>
<td>7.1‡</td>
<td>7.4‡</td>
<td>7.2§</td>
</tr>
</tbody>
</table>

L.S.D. *(P > 0.05) = 0.398

Texture scores

| 0                  | 8.8                    | 8.8†     | 8.8§        | 8.8§                                    | 8.8§                                     |
| 2                  | 8.6                | 8.6      | 8.7         | 8.7                                    | 8.6§                                     |
| 4                  | 8.5                 | 8.5      | 8.6†        | 8.6†                                    | 8.6†                                     |
| 6                  | 8.2                 | 8.2      | 8.4‡        | 8.4‡                                    | 8.4‡                                     |
| 8                  | 7.8                 | 7.8      | 8.3‡        | 8.3‡                                    | 8.3‡                                     |
| 10                 | 7.4                 | 7.1      | 7.8†        | 7.8†                                    | 7.8†                                     |
| 12                 | 6.2                | 6.2      | 7.1‡        | 7.1‡                                    | 7.1‡                                     |
| 14                 | 5.7                 | 5.5      | 7.1         | 7.4‡                                    | 7.2‡                                     |

L.S.D. *(P > 0.05) = 0.410

Overall acceptability scores

| 0                  | 9.0                    | 9.0      | 9.0†        | 9.0†                                    | 9.0†                                     |
| 2                  | 8.8                  | 8.8      | 8.8§        | 8.8§                                    | 8.8§                                     |
| 4                  | 8.6                 | 8.6      | 8.7         | 8.7‡                                    | 8.7‡                                     |
| 6                  | 8.5                 | 8.3      | 8.3‡        | 8.3‡                                    | 8.3‡                                     |
| 8                  | 8.3                 | 8.4      | 8.4‡        | 8.4‡                                    | 8.4‡                                     |
| 10                 | 7.9                 | 7.5      | 8.0         | 8.2‡                                    | 7.9‡                                     |
| 12                 | 5.4                | 5.2      | 7.4         | 7.4‡                                    | 7.4‡                                     |
| 14                 | 5.0                | 4.9      | 7.3         | 7.4‡                                    | 7.4‡                                     |

L.S.D. *(P > 0.05) = 0.404

L.S.D.: Least significant differences at 5% level. Mean with the same letter or letters are not significantly different.

** PA/PE: Polyamide / polyethylene film.
***LDPE: Low density polyethylene film without potassium sorbate or calcium propionate.

REFERENCES


إعداد البولي ثالث منخفض الكثافة المضادة للكائنات الحية الديفية:

- تأثيرها على تأثين سمك الرينة المدخن على البارد والمعم تحت تقريج أثناء تخزينه بالتدريج

أحمد توقيف العاقل
قسم الصناعات الغذائية - كلية الزراعة - جامعة القاهرة

تم إجراء هذا البحث لدراسة مدى أمانة استخدام بعض موارد الطهي والتخزين مثل أغذية البولي
ثالث منخفض الكثافة (سمك 180 ميكرون) بدون أو بإضافة موارد معتدلة للكائنات الحية الديفية مثل
سويت بونيتيم أو بروجيكت كلاسيكيات (2% و4% وزن/وزن) والبولي أميد. الزيت
وقد تركز الدراسة على معرفة الخصائص الميكانيكية (القدر على ربط الماء) والكيميائية (النشاطات الصلبة
والمواد التربوزية المثارة) ومربك ثانوي ميلاني (أمين، الأمين الكاثتي، الأحماض الكاسافية،
البيروكسيديت والثيوريدين والناكايودين) والمحيط (أسيت أمين). وتحتوي الثيوريتية
ed (العدد الكلي للميكروبات الحيوية والبكتيريا المحبة للبرودة) والحسي (عملية الرائحة والمواد
الماضية بصفة عامة) لسمك الرينة (Clupea harengus) المدخن على البارد والمعم تحت تقريج أثناء
التخزين على درجة حرارة 4 ملس.

أوضح النتائج أن أفضل المعالجات لسمك الرينة المدخن على البارد والمعم تحت تقريج في أغذية
البولي ثالث منخفض الكثافة المعتدلة على 4% سويت بونيتيم أو بروجيكت كلاسيكيات وذلك من حيث
الصفات الميكانيكية وفرصة الاصلاحية (قابل للإصلاح) بعد 10 أسابيع، بينما السمك المدخن في أغذية البولي
ثالث منخفض الكثافة المعتدلة على 2% ماء حافية (قابل للإصلاح) بعد 8 أسابيع. على المكس فبعد
المدخن والتخزين المكثف في مراكز الرينة المدخنة المغذية على البارد والمعم في كل من
البولي ثالث منخفض الكثافة (عيب ضارب) والبولي أميد البارد. الرينة أثناء السود في درجة
4 ملس في عمليتي الإنزال والتخزين بعد غذاء الأمعاء المكثف.

و على ذلك فإن التوصية بتعمل أغذية البولي ثالث منخفض الكثافة والمعالجة بالماء المدخن
المضادة للاحماء الديفية لحظ سمك الرينة المدخن على البارد والمعم تحت تقريج للمحافظة على درجة
الجودة وفترة مدة التخزين بالتدريج.