INTRODUCTION

The period 1975-1987 witnessed a market growth in the consumption and/or acceptance of surimi, minced and water-washed fish muscle tissues, in the form of manufactured items such as shellfish analogs. The estimated U.S. supply of surimi products increased from 2.7 to over 45.4 million kg during this same period (Vondruska, 1987).

Diverse species of fish have been used as raw material for surimi. Alaska pollock (Theragra chalcogramma) has been the primary fish used for crableg analogs but recently access to U.S. fishermen has been limited by the U.S. declaration of a 200-mile fisheries conservation zone. In addition, due to fishing pressure, alternative species such as Atlantic and Gulf menhaden (Brevoortia patronus and B. tyrannus, respectively) and Pacific whiting (Merluccius productus) have been investigated for surimi production. A growing interest in the use of dark-fleshed fish species for mince and surimi has developed during the so-called “Age of Engineered Seafoods”. Dark-fleshed, oily fish have been traditionally used for feed, oil and fertilizer production. With the increasing realization of the potential health benefits of engineered seafoods, red-fleshed, oily fish species are now regarded as possible sources of omega-3 unsaturated fatty acids in the diet (Lanier et al., 1988).

U.S. commercial fisheries landings for Gulf menhaden were 539,200 mt in 1993 (NMFS, 1993). Gulf menhaden are regarded as an industrial fish species. Before these fish can be utilized for human consumption, various problems need to be addressed. For instance, the improper on-board handling and storage of these fish, the lack of processing equipment suitable for heading and eviscerating small-sized fish, and the fact that these fish are fatty and dark-fleshed fish make them unacceptable for direct human consumption (Babbitt, 1986). However, due to its good gelling properties (Anonymous, 1989), menhaden has a potential as a protein...
matrix in which other seafoods could be combined to formulate various seafood products if flavor and color of the mince could be improved.

This work was to evaluate the effects of various pH buffered water-washing treatments on the Hunter color, hematin content, carotenoid content, TBA and, carbonyl values of menhaden mince and on moisture content of the gel.

MATERIALS AND METHODS

Preparation of Fish Mince

Gulf menhaden were caught off the Florida coast, chilled and held in slush ice (1 to 3°C) on the boat until processed (< 48h). The fish were transported to the Mississippi State University (MSU)/National Marine Fisheries (NMFS) Experimental Seafood Processing Laboratory at the Coastal Research and Extension Center, Pascagoula, MS. About 113 kg of fish were processed (Fig. 1) in June 9, July 23, and July 28, 1992 using commercial equipment: a Model M-072 Lapine Fish Heading Machine and a Model M-017 Lapine Fish Gutting Machine (Pisce s Industries, Ltd., Wells, MI).

The dressed fish exited the cutting area to enter a Model NDX13 deboner with 5-mm holes (Bidun Machine Construction Co., Ltd., Japan) to separate fish flesh from bones, skin and scales. The deboning process is also called a mincing process. A portion of the nonrefmed menhaden mince recovered from the dressed fish (about 14kg for each replication) was placed in wax coated storage boxes. The boxes (1.6 to 1.65kg) were stored in the walk-in freezer (-30°C) until they were shipped overnight on dry ice to the MSU Food Processing Laboratory, Miss. State, MS. The boxes were then stored in a walk-in freezer (-17°C) upon arrival on campus.

pH Washing Treatments

The mechanically deboned fish muscle (MDFM) was thawed at 4°C for 48 h before use.

Buffer Preparation

Phosphate buffers of pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, ± 0.2 and same ionic strength were prepared with analytical grade NaH₂PO₄·H₂O and Na₂HPO₄ (Fisher Scientific, Fair Lawn, NJ) by the procedures of Gomori (1955). Chilled distilled water was used to make the different buffer solutions which were then stored at 4°C until used.

Buffer and Water Washings

There were seven pH treatments, including a control (potable water, pH = 6.8). The washing process called for a batch wash system as follows:
- First cycle (4 parts of water to 1 part of mince): One kilogram of MDFM was
Figure 1, Menhaden Mince and Surimi Processing Flowchart.
washed with four liters of phosphate buffer at different pHs at 4°C. The mixture was manually stirred for 3 min, allowed to settle for 15 min and filtered through a double layer of fine cheese cloth. The water was pressed out by hand and floating particles of fat were removed.

- Second and third cycles (5 parts of water to 1 part of mince): The fish paste from the first cycle was washed with five liters of potable water at 4°C. In the 3rd cycle, 0.15% (w/w) of NaCl was added. After each cycle, the mixture was hand stirred for 3 min, allowed to settle for 5 min and then filtered through a double layer of fine cheese cloth. The fish paste was further pressed using a Model YS200 Screw Dehydrator (Bidun Machine Construction Co., Ltd., Japan) to reduce the moisture content. Washed meat was weighed to separate samples used for different chemical analyses from the one used for the preparation of the gels.

### Preparation of Surimi Gels

The fish paste was mixed with 4% sucrose (commercial granulated refined sugar), 4% sorbitol (Neosorb 20/60, Roqueete Corporation, Gurnee, IL) and 0.25% sodium tripolyphosphate (BK-Landenburg, Corp., Cresskill, NJ) on a w/w basis in a household mixer (Kitchenaid, Hobart Manufacturing Company, Troy, OH) (Fig. 1). The total mixing time was 3 min at speed 4; subsequently, the surimi was stored in a 0.076-mm polyethylene bag at -20°C until used.

The surimi was thawed overnight in a refrigerator then chopped with 2% (w/w) NaCl in a silent cutter (Model VCM40, Hobart Manufacutring Company, Troy, OH) for 3 min. An additional 6 min of chopping was performed with a sufficient amount of ice-chilled water (2 ± 1°C) to adjust the moisture content to 78%. The chopped paste was stuffed into 3cm diameter cellulose casings, and cooked at 90°C for 40 min in a water bath. The heat induced surimi gels were cooled in running tap water for 20 min. The prepared gels were allowed to equilibrate to room temperature overnight and then cut into 3 cm dia. and 2 cm long cylindrical shapes for color measurements.

### Chemical Analysis

The fish paste saved for chemical studies was stored for 24 h at 4°C prior to analysis.

Total carotenoid pigments were determined calorimetrically (OD₄₅₀) after extraction on 25g of fish paste, as described by Saito and Regier (1970). The OD at 460 nm was multiplied by a factor of 2 to express the results as µg carotenoids/g sample. The concentration of the carotenoids were determined at pH 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and control.

The hematin content of the menhaden fish was determined on 5g of fish paste by the alkaline hematin method as described by Karlsson and Lundstrom (1991).
Hematin concentration, expressed as ppm hematin/g sample, was calculated using the following equation:  

$$\text{ppm} = \frac{5}{50} \times [97.261(\text{OD}_{700}) - 0.0751]$$

Oxidative deterioration of the lipid components (rancidity) was evaluated on log sample using the 2-thiobarbituric acid test of Tarladgis et al. (1960). The optical density was read at 538 nm and readings multiplied by a factor of 7.8 to express the TBA reactive substances (TBARs) results as mg of malonaldehyde/kg sample (Sinnhuber and Yu, 1958).

The carbonyl content (moles/g) was determined by the method for trace quantities of carbonyl compounds as described by Siggia (1963). Results were converted to µmole/g by multiplying by 10^6.

Moisture content of unwashed menhaden mince, pH treated menhaden paste and the control were determined by method 950.46, drying under vacuum at 95 to 100°C (AOAC, 1990). Values were reported as percent moisture on a wet weight basis.

**Color Measurement**

Hunter "L" (lightness), "a" (+ indicating redness) and "b" (+ indicating yellowness) values were measured by a Hunter Labscan 6000 0/45° Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA). The instrument was calibrated using a white Hunterlab color standard tile no. LS- 13601 (L=90, a_0= 0.16 and b_0=0.48). The fish samples (at room temperature) were placed on to a 60x15mm (dia x ht) clear Pyrex© culture petri dish cover and over a 2 in. port. Hunter "I", "a", and "b" values were recorded for all pH treatments of both menhaden paste and heat-induced surimi gel.

The hue angle, \(\tan^{-1}(b/a)\) and total color difference \(AE = [L-L_0]^2 + (a-a_0)^2 + (b-b_0)^2]^{1/2}\) were calculated for menhaden mince.

**Statistical Analysis**

All data were analyzed using a randomized complete block design with three replications (blocks). Blocks consisted of three batches of menhaden fish processed June 9, July 23 and July 28 1992, respectively. The treatments consisted of unwashed mince, paste/gel washed with tap water (control), and paste/gel washed with phosphate buffers at pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Analyses were performed using the GLM procedures of the Statistical Analysis System (SAS, 1985). Means were separated where significant differences were found using least significant differences (LSD) test (SAS, 1985) at the 5% level of significance.
RESULTS AND DISCUSSION

Chemical Analysis

There were no differences (P>0.05) in carotenoids content, on a dry basis, due to wash treatments (Table 1). Due to their aliphatic and aliphatic-cyclic molecular structure composed of carbon isoprene groups, carotenoids are fat-soluble pigments. Consequently, aqueous washing treatments do not extract these pigments.

Total heme pigment extraction was affected (P>0.05) by washing medium pH (Table 1). Washing with phosphate buffers of pH 8.0 or lower resulted in lower (P>0.05) hematin than unwashed products. The pigment content decreased by 74%, from 1.07 ppm in the unwashed fish mince to 0.27 ppm in the tap water washed sample. Dawson et al. (1988) reported that heme pigments are more soluble in low salt concentrations (Froning and Niemann, 1988), possibly facilitating their removal by the washing solution.

There were no differences (P>0.05) in TBARs due to wash treatments. The TBARs varied between 8.40 for unwashed mince and 4.53 mg of malonaldehyde/kg for mince washed with pH 5.0 water (Table 1). These values are considered to be high for frozen seafoods (Sinnhuber and Yu, 1958) and may result in unacceptable products by consumers. Disruption of muscle membrane system caused by deboning was postulated to increase the rate of lipid oxidation by exposing labile lipid constituents to oxygen (Sato and Hegarty, 1971). Hall (1987) stated that a rupture of the organized cellular structure brought together lipids, catalysts and enzymes involved in lipid oxidation.

Nakayarna and Yamamoto (1977) reported that rancidity was detected at TBA values of 1.50 and 3.00 in different fish species. However, malonaldehyde is not the only end product of lipid peroxidation (Buege and Aust, 1978). Furthermore, Slabyj and True (1978) reported that high TBA values did not necessarily reflect rancidity in some samples.

There were differences (P<0.05) in carbonyl values due to wash treatments (Table 1). The application of water washing to raw fish mince decreased carbonyl values in mince. Carbonyl values decreased by 50% from 0.463 in the unwashed raw fish mince to 0.233 µmole/g in the tap water-washed sample but were not different (P>0.05) among washed minces.

Carbonyl values reported in the fish mince washed with a pH 5.0 phosphate buffer were usually higher than in those washed with alkaline pHs. This may be attributed to lipid oxidation by nonheme proteins (proteins associated with free inorganic forms of iron, copper, or cobalt), which have been reported to be responsible for about two-thirds of oxidation (Decker and Schanus, 1986) and found to be pH sensitive; the catalytic effect of the iron being higher at acidic pH values.
Table 1. Mean carotenoids, hematin, 2-thiobarbituric acid reactive substances (TBARs) and, carbonyl values of menhaden mince as affected by different washing treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoids (dry basis) (ppm)</th>
<th>Hematin (dry basis) (ppm)</th>
<th>Carbonyls (µmole/g)</th>
<th>TBARs (mg malonaldehyde/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>1.07 NS</td>
<td>0.27 bc</td>
<td>0.320 ab</td>
<td>4.53 NS</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>0.70</td>
<td>0.17 c</td>
<td>0.270 bc</td>
<td>6.73</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>0.87</td>
<td>0.23 bc</td>
<td>0.170 bc</td>
<td>5.90</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>0.90</td>
<td>0.17 c</td>
<td>0.157 c</td>
<td>5.00</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>0.90</td>
<td>0.67 ab</td>
<td>0.203 bc</td>
<td>4.77</td>
</tr>
<tr>
<td>pH 10.0</td>
<td>1.03</td>
<td>0.63 ab</td>
<td>0.150 c</td>
<td>6.37</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.57</td>
<td>0.27 bc</td>
<td>0.233 bc</td>
<td>6.67</td>
</tr>
<tr>
<td>Unwashed</td>
<td>0.63</td>
<td>1.07 a</td>
<td>0.463 a</td>
<td>8.40</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>0.83</td>
<td>0.43</td>
<td>0.246</td>
<td>6.05</td>
</tr>
<tr>
<td>CV (%)</td>
<td>45.24</td>
<td>64.17</td>
<td>36.01</td>
<td>22.59</td>
</tr>
<tr>
<td>SEM</td>
<td>0.14</td>
<td>0.08</td>
<td>0.078</td>
<td>1.87</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.66</td>
<td>0.49</td>
<td>0.155</td>
<td>2.39</td>
</tr>
</tbody>
</table>

abc- Means within columns not followed by a common letter differ (P<0.05).
NS- No significant differences
CV - Coefficient of Variability
SEM - Standard error of the mean
LSD - Least significant differences at 5% level of probability
Labuza (1971) suggested that the protein part of hemoprotein molecules normally hinders the catalytic function of the iron, and in the denaturation of the protein part of hemoprotein molecules could expose iron to lipids.

Mean moisture values of menhaden mince and menhaden gel as affected by different washing treatments are shown in Table 2. Menhaden mince from meat washed with water pH 8.0, 9.0 and 10.0 had higher (P<0.05) moisture than those washed with low pH buffer or tap water. Moisture content for any washed mince sample was higher (P<0.05) than for the unwashed mince sample. No differences (P>0.05) in gel moisture were found, since this was made to a target 78% moisture (Fig. 1).

It was noticed that water removal from the washed menhaden mince during the dewatering step was particularly difficult as the water pH increased (data not shown). Ball et al. (1984) reported a similar direct relationship between increased washing solution pH and increased moisture content in washed poultry meat. The increase of water binding properties of muscle proteins was attributed to an adjustment of pH away from the protein isoelectric point, in mechanically deboned poultry meat (Dawson et al., 1988).

Color

Hunter "L", "a", hue value, and, total color difference (AE) of menhaden mince of washed treatments was different (P<0.05) from the unwashed. The most dramatic effect of water washings was a 84% reduction in redness ("a" value), from 4.49 to 0.72 (Table 3). The fish mince Hunter L’ value was reduced by 10 units after washing, but wash pH did not have any effect.

Hue angle values (Table 3) increased from 68.74 in the unwashed fish mince to 86.97 in the tap water washed sample. There was no additional increase in the hue values when different phosphate buffers were used. As the red color was removed, the fish mince decreased in darkness, and hues other than red tended to predominate. Total color difference (AE) values between untreated and treated samples decreased (P<0.05) with washing.

The changes in color of the washed mechanically deboned fish mince during the cooking step could be attributed to the denaturation of any remaining myoglobin to denatured metmyoglobin (Francis and Clydesdale, 1975). Fogg and Harrisson (1975) reported that myoglobin in pure solution denatured when heated to 85°C. Francis and Clydesdale (1975) stated that upon cooking, salmon flesh was lighter in color and hue shifted from red to orange-red. Contamination of menhaden mince with skin, bone and scale indicated too high deboner belt pressure and an improper feeding of the deboner. Fish fed skin side down were used, forcing more bone and scale through the drum perforations along with the fish mince. This outcome could have been avoided by adjusting the belt pressure of the deboner and orienting the split fish so that the deboner perforated drum was in direct contact with the white muscle (bone
Table 2. Mean moisture values of menhaden gel as affected by different washing treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mince Moisture (%)</th>
<th>Gel Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>81.30 b</td>
<td>77.90 ab</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>81.63 b</td>
<td>78.30 ab</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>84.77 ab</td>
<td>79.20 a</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>85.47 a</td>
<td>79.23 a</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>86.60 a</td>
<td>79.77 a</td>
</tr>
<tr>
<td>pH 10.0</td>
<td>86.30 a</td>
<td>78.67 a</td>
</tr>
<tr>
<td>Tap water</td>
<td>78.63 b</td>
<td>77.13 b</td>
</tr>
<tr>
<td>Unwashed</td>
<td>71.87 c</td>
<td>N/A</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>82.07</td>
<td>78.54</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.24</td>
<td>1.14</td>
</tr>
<tr>
<td>SEM</td>
<td>3.37</td>
<td>0.80</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>3.22</td>
<td>1.57</td>
</tr>
</tbody>
</table>

abc - Means within columns not followed by a common letter differ (P<0.05).
N/A - Data not available
CV - Coefficient of Variability
SEM - Standard error of the mean
LSD - Least significant differences at 5% level of probability.
Table 3. Mean Hunter “L”, “a”, hue, and AE color values of menhaden mince as affected by different washing treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L</th>
<th>a</th>
<th>Hue</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>51.92 a</td>
<td>0.11 bc</td>
<td>89.70 a</td>
<td>39.35 b</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>51.70 a</td>
<td>0.33 bc</td>
<td>88.61 a</td>
<td>39.59 b</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>51.44 a</td>
<td>0.16 bc</td>
<td>89.53 a</td>
<td>39.73 b</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>52.98 a</td>
<td>0.28 bc</td>
<td>88.42 a</td>
<td>38.15 b</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>53.27 a</td>
<td>-0.06 c</td>
<td>90.68 a</td>
<td>37.97 b</td>
</tr>
<tr>
<td>pH 10.0</td>
<td>51.95 a</td>
<td>0.13 bc</td>
<td>89.86 a</td>
<td>39.22 b</td>
</tr>
<tr>
<td>Tap Water</td>
<td>51.28 a</td>
<td>0.72 b</td>
<td>86.97 a</td>
<td>40.04 b</td>
</tr>
<tr>
<td>Unwashed</td>
<td>41.41 b</td>
<td>4.49 a</td>
<td>68.74 b</td>
<td>49.97 a</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>50.74</td>
<td>0.77</td>
<td>86.56</td>
<td>40.50</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.18</td>
<td>54.27</td>
<td>2.55</td>
<td>3.87</td>
</tr>
<tr>
<td>SEM</td>
<td>2.60</td>
<td>0.19</td>
<td>4.86</td>
<td>2.46</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>2.82</td>
<td>0.76</td>
<td>3.86</td>
<td>2.75</td>
</tr>
</tbody>
</table>

\(^1\text{Hue} = \text{Tan}^{-1} (b/a)\)

\(^2\Delta \text{E} = \text{Total color difference} = [(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2]^{1/2}\)

abc - Means within columns not followed by a common letter differ (P < 0.05).
CV - Coefficient of Variability
SEM - Standard error of the mean
LSD - Least significant differences at 5% level of probability.
side). These adjustments are essential to control the quantity of dark muscle incorporated as well as skin, bone and scale.

CONCLUSIONS

Washing with tap water or pH-buffered water (acid or alkaline) resulted in a lighter (P<0.05) color mince than unwashed product. However, there were no differences (P>0.05) in washing treatment on color of mince and gel made from menhaden. There was no effect (P>0.05) of washing treatment on carotenoids or TBARs, but there were (P<0.05) on hematin and carbonyl values of the mince.

ACKNOWLEDGMENTS

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REFERENCES


The shelf life and safety of refrigerated fish and fish products are dictated by the presence of food spoilage and pathogenic microorganisms (Vilemure et al., 1986; Ingham, 1989; Harrison et al., 1991; Kim and Heamsberger, 1994). Temperature abuse during handling, transportation, and storage of fish without additional preservation methods allows for proliferation of these microorganisms (Barnett et al., 1982; Fey and Regenstein, 1982; Josephson et al., 1985; Scott et al., 1986; Baker et al., 1990). Growth of undesirable spoilage bacteria in refrigerated fish causes deterioration of keeping quality and reduces consumer acceptance.

Efforts to enhance the quality and safety of refrigerated foods have been primarily directed to preventing the growth of or to destroy aerobic spoilage bacteria and foodborne pathogens during storage and handling by using combinations of food additives and/or biopreservatives (Gilliland and Ewell, 1983; Lindgren and Dobrogosz, 1989). Several researchers have suggested that biopreservatives such as lactic acid bacteria or their metabolites in refrigerated foods have the ability to suppress aerobic bacteria that cause food spoilage (Raccach and Baker, 1978; Schaaack and Marth, 1988; Laroia and Martin, 1990; Modler et al., 1990; Hughes and Hoover, 1991; Ray, 1992).

Phosphates have broad spectrum antimicrobial activity and are used for improving the microbiological quality of muscle foods and to improve shelf life (Molins, 1991). Molins (1991) reported that changes in pH induced by phosphate addition may play an important role in the ability of these compounds to chelate metal ions essential in bacterial metabolism and growth. He suggested that, in general, gram-positive bacteria are more susceptible to inhibition by various pyro- and polyphosphates than are gram-negative bacteria. Although there has been limited
work on combining acetates and phosphates in meat products (Mendonca et al., 1989) as antimicrobial surface treatments, this has not been studied with fish.

Lindgren and Dobrogosz (1989) observed that a combination of Lactobacillus leuteri and 250 mM glycerol inhibited aerobic spoilage bacteria in herring fillets stored in N₂ at 5°C for 6 d. Raccach et al. (1979) and Schaack and Marth (1988) showed similar effects with lactic acid bacteria on food spoilage bacteria and pathogens in poultry meat and skim milk, respectively. Use of Pediococcus cerevisiae (Accel®) and Lactobacillus plantarum (Lactacel®) in refrigerated deboned poultry meat decreased the microbial population, reduced off-odor, and extended shelf-life by 2 d (Raccach et al., 1979). Kim and Heamsberger (1994) reported that combination of Lactococcus lactis ssp. cremoris ATCC 19257 with sodium acetate and potassium sorbate effectively inhibited growth of gram-negative bacteria on refrigerated catfish fillets. Bifidobacteria may control food spoilage bacteria and foodborne pathogens through production of lactic and acetic acids as well as other antibiotic substances (Laroia and Martin, 1990; Modler et al., 1990; Hughes and Hoover, 1991; Ray, 1992). Previous work in our laboratories has shown that sodium acetate is effective in suppressing the growth of aerobic spoilage bacteria on refrigerated catfish fillets (Kim and Heamsberger, 1994). Because there are no studies on the combined effect of sodium acetate with phosphates or bifidobacteria as an antimicrobial treatment of fish or fish products, the present study was designed to evaluate shelf-life of refrigerated (4°C) channel catfish fillets surface-treated with sodium acetate, monopotassium phosphate and/or selected bifidobacteria.

MATERIALS & METHODS

Preparation of bifidobacteria cultures

Bifidobacterium infantis ATCC 15697 and B. longum ATCC 15707 were obtained from the American Type Culture Collection (Rockville, MD). B. adolescentis 9H Martin was obtained from the culture collection of Dr. J. H. Martin, Mississippi State University. Stock cultures were maintained in sterile skim milk medium (SM; 10% skim milk, 0.5% yeast extract, and 0.5% glucose) at 37°C for 24 h in an anaerobic jar (Gas-Pak; BBL). For fish inoculation, the cultures were prepared by three successive 1: 10 transfers at 24-h intervals into fresh SM incubated as above. Bifidobacteria cultures in SM (6.0 x 10⁷ to 1.0 x 10⁸ CFU/ml) were added at a given percentage (V/W) to fresh catfish fillets as described below. Final SM pH was 4.4-4.8.

Catfish preparation and treatment

Fresh channel catfish (Ictalurus punctatus) fillets were obtained from a commercial source, transported to MSU on ice, and used within 3 h. For each treatment, 2 kg of fillets (average weight of 110 g per fillet) were placed in a precleaned (Jetson General Purpose Cleaner, Diversy Group, Cincinnati, OH) and sanitized (hot water at 88°C) tumbler (Polymaid Model SS 350, Lax-go, FL) at room temperature. Food preservatives were added to the tumbler and fillets were tumbled
for 15 min at 18 r-pm. Untreated controls were tumbled without preservatives. The tumbler was cleaned and sanitized between treatments. Duplicate experimental trials consisted of the following treatments: (a) 0.0, 0.25, 0.5, 0.75, or 1.0% (W/W) sodium acetate (SA; Fisher Scientific Co., Norcross, GA), (b) 0.0, 0.25, 0.5, 0.75, or 1.0% (W/W) monopotassium phosphate (MKP; FMC Corp. Philadelphia, PA), (c) 0 or 2.5% (V/W) *B. adolescentis*, *B. infantis*, or *B. longum* (V/W), (d) 0.5% SA and 0.1, 0.2, 0.3, or 0.4% MKP, (e) 0.4% MKP and 0.3, 0.5, or 0.7% SA, (f) 0 or 2.5% of *B. adolescentis*, *B. infantis*, or *B. longum* combined with 0 or 0.5% SA, (g) 0 or 0.5% SA combined with 1.5%, 2.5%, 3.5% or 4.5% *B. infantis*, and (h) 0 or 0.5% SA combined with 2.5% *B. infantis* incubated for 24, 48, or 72 h before addition. Treated catfish fillets were removed from the tumbler and stored individually in “Ziploc®” (DowBrands L.P., Indianapolis, IN) bags at 4°C. Storage bag gas transmission rate is 1.65 cc/cm² per mil in 24 h at 1 atm. Microbiological analysis was performed for each treatment over a 12 d storage period. Selected treatments were scheduled for pH measurements and sensory evaluations during storage.

**Microbiological analyses**

Each fillet was weighed and 0.1% sterile peptone water was added to make a 1:1 dilution (W/V). A Stomacher Lab Blender 400 (Tekmar, Cincinnati, OH) was used to homogenize the specimen for 2 min and then appropriate serial dilutions were made for spiral plating. Total aerobic plate counts (APC) were determined by duplicate spiral-platings (Spiral System, Inc., Bethesda, MD) on standard plate count agar (Difco Detroit, MI) incubated at 30°C for 2 d before counting (Swanson et al., 1992). APC values were expressed as mean log., CFU/g for 2 fillets per treatment per sampling day. Generation times were calculated as described elsewhere (Marshall and Schmidt, 1988).

Bifidobacteria were enumerated by pour plating appropriate serial dilutions in 0.1% peptone water with neomycin-paromomycin- nalidixic acid-lithium chloride agar (Teraguchi et al., 1978; Laroia and Martin, 1991) followed by incubation at 37°C for 48 h in an anaerobic jar (Gas-Pak; BBL) prior to counting colonies.

**Measurement of pH**

Fillet pH was measured with a standardized pH meter (Accumet®, Model 50, Fisher) by placing a surface electrode directly onto the fillet surface. Mean pH values were reported as the average of quadruplicate readings for each duplicate fillet.

**Sensory evaluation**

Sensory evaluation of samples was performed by an eight member untrained panel. Uncooked odor and appearance of treated fillets were evaluated every 3d during storage at 4°C. Samples were warmed to room temperature prior to analyses. Treated samples were judged against a fresh control (fresh fillets were used each analysis day) which was assigned a score of 5. Samples liked less than the control were scored 1 to 4, where 1 = most disliked. Samples liked more than the control were scored 6 to 9, where 9 = most liked. Untreated fillets also were compared with
untreated fresh fillets and treated fillets. Two fillets per treatment per sampling day were analyzed.

**Statistical analyses**

APC, pH, and sensory data were analyzed using ANOVA, and means were separated by the least significant difference test (SAS, 1992).

**RESULTS & DISCUSSION**

**Combined effect of sodium acetate and monopotassium phosphate**

APC of refrigerated (4°C) catfish fillets were affected by increasing levels of SA (Fig. 1). Use of 0.75% and 1.0% SA significantly lowered (P<0.05) initial APC by 0.6-0.7 log units compared to the control. Generation times (GT) of aerobic bacteria on fillets treated with SA were significantly higher (P<0.05) than the control (Fig. 2). Spoilage of most muscle foods is thought to occur when APC reach 10⁷ CFU/g or greater (Ayers, 1960). Therefore, SA treatments, at 0.75% or greater, of fillets could increase shelf-life (as measured by APC) by 6 d under present experimental conditions. These results confirm previous work that demonstrated the antimicrobial potential of SA on catfish fillets, where 0.5-1.0% SA suppressed growth of gram-negative bacteria at 4°C for at least 6 d (Rim and Hearnsberger, 1994).

MKP in refrigerated catfish fillets had no effect on APC values (results not shown) or generation times (Fig. 3). Treatment with MKP lowered fillet pH values by 0.5-0.9 units (results not shown). Kim and Heamsberger (1994) reported that the combined antimicrobial effects of SA, potassium sorbate, and lactic acid bacteria in refrigerated catfish fillets were not pH related. Results of the present catfish study are consistent with previous work by Molins (1991) who reported that other phosphates (sodium acid pyrophosphate or sodium tripolyphosphate) did not improve the microbiological quality of cooked, vacuum-packaged bratwurst held at 5°C for 7 d.

Combining MKP and SA effectively inhibited growth of aerobic microorganisms (Figs. 3 and 4). All samples treated with the combination had significantly (P<0.05) lower APC after 3 d of storage than the control. Combining MKP with 0.5% SA reduced numbers during latter stages of storage more than did SA alone (results not shown), which was reflected by increased generation times with increasing MKP concentration (Fig. 3). Conversely, when MKP concentration remained at 0.4%, no differences (P>0.05) in inhibition were observed with increasing Concentrations of sodium acetate (0.3-0.7%) (Fig. 4). Results indicate that the interaction between MKP and SA is likely additive.

Although MKP alone had no inhibitory effect, it may cause a decline of water activity values in treated fish fillets (Synder and Maxcy, 1979) and have indirect antimicrobial effects due to chelation of metal ions essential for bacterial metabolism and cell integrity (Scott et al., 1986). Microbial spoilage of fish is caused by gram-
Fig. 1. Total aerobic bacterial counts on catfish fillets treated with sodium acetate (SA) during storage at 4°C.

Fig. 2. Generation times of aerobic bacteria on catfish fillets treated with sodium acetate (SA) or monopotassium phosphate (MKP) and stored at 4°C. Means within the same cluster having the same letter are not significantly different (P>0.05).
Fig. 3. Generation times of aerobic bacteria on catfish fillets treated with different concentrations of monopotassium phosphate (MKP) combined with 0.5% sodium acetate (SA) and stored at 4°C. Means with the same letter are not significantly different (P>0.05).

Fig. 4. Generation times of aerobic bacteria on catfish fillets treated with different concentrations of sodium acetate (SA) combined with 0.4% monopotassium phosphate (MKP) and stored at 4°C. Means with the same letter are not significantly different (P>0.05).
Fig. 5. Aerobic plate counts of catfish fillets treated with Bifidobacterium infantis culture (BI) and sodium acetate (SA), either alone or combined, during storage at 4°C.

Fig. 6. Generation times of aerobic bacteria at 4°C on catfish fillets treated with Bifidobacterium infantis Culture (BI) and sodium acetate (SA), either alone or combined. Means with the same letter are not significantly different (p>0.05).
negative bacteria such as Pseudomonas spp. (Herbert et al., 1971; Herbert and Shewan, 1976). SA inhibits growth of gram-negative bacteria on refrigerated (4°C) catfish fillets (Kim and Hearnsberger, 1994), while gram-positive bacteria are more susceptible to inhibition by MKP than are gram-negative bacteria (Molins, 1991). This microbial specificity demonstrates that SA is a better chemical treatment than MKP to extend shelf-life of catfish. However, results indicate that a combination of SA and MKP may prolong the microbiological shelf life of catfish at 4°C by keeping counts below 10⁷ CFU/g.

**Combined effect of sodium acetate and bifidobacteria**

The effects of various concentrations and species of BIF and SA on the shelf-life of refrigerated catfish fillets were studied on the basis of aerobic plate counts (APC) and sensory data. When a 2.5% culture of *B. infantis* was used alone, little impact on APC was noted with the exception of a significant (P<0.05) extension of the lag phase (Fig. 5). Once growth began, GT of aerobic microorganisms on fillets treated with *B. infantis* were not significantly different (P>0.05) from control fillets (Fig. 6). Similar (P>0.05) extended lag phases and GT were noted for fillets treated with 2.5% cultures of *B. adolescentis* or *B. longum* (results not shown). Hence, further results only will report data from *B. infantis* treatments. Lag phase increases of psychrotrophic populations were observed in ground poultry treated with *Lactobacillus plantarum* or *Pediococcus cerevisiae* (Raccach et al., 1979). It was assumed that inoculated BIF do not grow on refrigerated fish fillets and the extension of lag phase may be due to compounds produced during culturing, i.e. acetic and/or lactic acid, that were inhibitory to aerobic spoilage bacteria (Gilliland and Speck, 1975; Ray, 1992). Other reasons for inhibited aerobic growth might include nutrient competition, unfavorable changes in oxidation reduction potential, or production of other antimicrobial compounds.

Fillets treated with 0.5% SA had APC significantly lower and GT significantly higher (P<0.05) than control or BE-treated fillets (Fig. 5 & 6). The microbiological shelf-life (i.e. time to reach APC of 10⁷ CFU/g or greater (Ayers, 1960)) of SA-treated fillets was prolonged by 3 d compared to untreated and BIF-treated fillets. Combining 2.5% BIF with 0.3% SA resulted in significant (P<0.05) lag phase extension and increased GT values approximately 8 h higher than controls (results not shown). No differences (P>0.05) among species was noted. When 2.5% *B. infantis* was combined with 0.5% SA, significant (P<0.05) decreases in APC and increases in GT were observed (Fig. 5 & 6). No significant difference (P>0.05) in GT was seen between this treatment and 0.5% SA alone, resulting in a 3d microbiological shelf-life extension. *B. infantis* behaved similarly (P>0.05) as the other two cultures when combined with 0.5% SA (results not shown). The combined treatments could increase GT by approximately 20 h. A previous report on SA combined with potassium sorbate or lactic acid culture has attributed antimicrobial effects primarily to SA (Kim and Hearnsberger, 1994). The present study shows that an additive interaction occurs when BIF are combined with SA.
Efforts to increase antimicrobial activity of the BIF-SA treatment proved unsuccessful. Increasing the amount of *B. infantis* culture had no influence \( (P>0.05) \) on APC (results not shown). Likewise, increasing the amount of time the *B. infantis* culture was incubated prior to application had no influence \( (P>0.05) \) on APC (results not shown). Reddy et al. (1970) reported that addition of 10% cultures of *Leuconostoc citrovurum* was effective in inhibiting the growth of aerobic spoilage bacteria in ground beef stored at 7°C for 7 days. In a later study, Reddy et al. (1983) found that when cultivation was prolonged from 24 to 48 h, growth and antibiotic production of *Lactobacillus bulgaricus* in skim milk was enhanced.

**Catfish pH and sensory scores**

Addition of SA decreased pH of fillets by 0.2-0.3 units (results not shown). Fillets treated with both SA and MKP had initial pH values 0.25-0.3 units lower than the control (Table 1).

**Table 1.** Mean pH values of catfish fillets treated with combinations of sodium acetate (SA) and monopotassium phosphate (MKP) during storage at 4°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.45a</td>
<td>6.40a</td>
<td>6.45a</td>
<td>6.30a</td>
<td>6.40a</td>
</tr>
<tr>
<td>0.3% SA-0.4% MKP</td>
<td>6.15b</td>
<td>6.25a</td>
<td>6.25a</td>
<td>6.25a</td>
<td>6.30a</td>
</tr>
<tr>
<td>0.5% SA-0.4% MKP</td>
<td>6.15b</td>
<td>6.25a</td>
<td>6.30a</td>
<td>6.30a</td>
<td>6.35a</td>
</tr>
<tr>
<td>0.7% SA-0.4% MKP</td>
<td>6.20b</td>
<td>6.25a</td>
<td>6.30a</td>
<td>6.35a</td>
<td>6.40a</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts are significantly different \( (P<0.05) \).

This decline was attributed to SA. After 3 d of storage, pH values changed little and did not differ \( (P>0.05) \) from the control. Initial pH values of fillets treated with 0.5% SA and 2.5% BIF, either alone or combined, were 0.4-0.5 units lower than untreated control fillets (results not shown). Other work has shown that SA-treated catfish fillets do not necessarily have lower pH values than controls, but remain inhibitory to growth of aerobes (Kim and Heamsberger, 1994). Hence, inhibitory results seen in the present study are likely due to changes in pH and to the action of SA itself.

Sensory results of SA-MKP experiments clearly showed panel preference for treated samples over stored untreated controls (Table 2). Sensory assessment of untreated controls was halted after 3 d of storage because of excessive off odor. Fillets treated with SA alone or combined with MKP had odor scores that were statistically indistinguishable \( (P>0.05) \) from fresh controls for up to 9 d (Table 2). However, appearance scores of fillets were significantly lower \( (P<0.05) \) after 3 d, likely due to a brownish and watery appearance (Table 2). Microbial spoilage of fish muscle is due to production of volatile nitrogen compounds, volatile acids, \( \text{H}_2\text{S} \), and mercaptans (Miller et al, 1973; Reineccius, 1977). Sensory data indicate that SA
concentrations up to 1% could be used on catfish without contributing a typical acetic acid (vinegar) odor.

Table 2. Mean sensory evaluation scores of catfish fillets treated with sodium acetate (SA) and monopotassium phosphate (MKP), either alone or combined, during storage at 4°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Odor Score</th>
<th>Appearance Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 3 6 9 12</td>
<td>0 3 6 9 12</td>
</tr>
<tr>
<td>Fresh Control</td>
<td>5.0a 5.0a 5.0a 5.0a 5.0a</td>
<td>5.0ab 5.0a 5.0a 5.0a 5.0a</td>
</tr>
<tr>
<td>1.0% SA</td>
<td>5.2a 4.5a 4.4a 4.0a 3.7b</td>
<td>5.4a 5.2a 4.3b 3.4b 3.6b</td>
</tr>
<tr>
<td>0.3% SA-0.4% MKP</td>
<td>4.8a 5.0a 4.0a 3.8b 3.0b</td>
<td>4.6b 4.8a 4.4b 3.3b 3.9b</td>
</tr>
<tr>
<td>0.5% SA-0.4% MKP</td>
<td>4.4a 4.6a 4.7a 5.2a 2.9b</td>
<td>5.3a 4.6a 4.1c 4.0a 3.5b</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>5.0a 1.5b ND ND ND</td>
<td>5.0ab 2.9b ND ND ND</td>
</tr>
</tbody>
</table>

Means within the same column with different superscripts are significantly different (P<0.05).
ND: Not determined.
Note: Fresh control assigned 5, with samples liked less than the control scored 1 to 4 (1 = most disliked) and samples liked more than the control scored 6 to 9 (9 = most liked).

Sensory scores of SA-BIF experiments indicate that treated fillets were in the "liked less" to "typical" category in appearance and odor compared to fresh control fillets (Table 3). Stored untreated control fillets were considered organoleptically unacceptable (associated with spoiling fish) by 6 d of storage and were discontinued from sensory evaluation. Odor and appearance of fillets treated with 0.5% SA and 2.5% B. *infantis* either alone or combined, was the same (p>0.05) as fresh fillets up to 6 d. Fillets treated with SA and BIF or SA alone were rated higher than fillets with BIF alone after 9 d. APC of fillets treated with BIF alone rapidly increased after 3 d of storage, yet the fillets were not considered spoiled by the panel until after 6 d.

**CONCLUSIONS**

SA alone or combined with MKP or BIF inhibited growth of aerobic spoilage bacteria on refrigerated (4°C) catfish fillets. MKP or BIF had little activity alone, but could increase effectiveness of SA. Therefore, use of MKP or BIF alone has trivial value in extending shelf-life of refrigerated catfish fillets. SA alone or combined with MKP or BIF is recommended to extend the microbiological shelf-life of refrigerated catfish fillets with high (10^6 CFU/g) initial microbial loads.
Table 3. Mean sensory evaluation scores of catfish fillets treated with sodium acetate (SA) and *Bifidobacterium infantis* culture (BI), either alone or combined, during storage at 4°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Odor Score</th>
<th>Appearance Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Fresh Control</td>
<td>5.0a</td>
<td>5.0a</td>
</tr>
<tr>
<td>2.5% BI</td>
<td>4.7a</td>
<td>4.8a</td>
</tr>
<tr>
<td>0.5% SA</td>
<td>4.6a</td>
<td>4.4a</td>
</tr>
<tr>
<td>2.5% BI-0.5% SA</td>
<td>5.4a</td>
<td>5.0a</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>5.0a</td>
<td>5.1a</td>
</tr>
</tbody>
</table>

a-c Means within the same column with different superscripts are significantly different (P<0.05).
ND: Not determined.
Note: Fresh control assigned 5, with samples liked less than the control scored 1 to 4 (1 = most disliked) and samples liked more than the control scored 6 to 9 (9 = most liked).

ACKNOWLEDGMENTS

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REFERENCES


Effect of Tray Design and Packaging Type on Microbial Growth, Surface pH and Sensory Ratings of Refrigerated Channel Catfish Fillets

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INTRODUCTION

Gram negative spoilage bacteria such as *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, and *Cytophaga* species are the major contributors to deterioration of fish and seafood products (Farber, 1991). Modified atmosphere packaging (MAP) using mixtures of carbon dioxide, oxygen, nitrogen, or other gases has been studied extensively to prolong shelf-life of fishery products (Silva et al, 1993; Wang and Oglydziak, 1986; Stenstrom, 1985; Gray et al., 1983; Parkin et al., 1981; Wolfe, 1980) and thus, extend marketable life of the products. Studies by Gray et al. (1983) in fish products and Sander and Soo (1978) in chicken products, showed that MAP systems were better in extending shelf-life than ice or vacuum packaging systems.

Carbon dioxide is the major gas used in MAP because of its effectiveness in retarding microbial growth in refrigerated, perishable foods. It is especially effective against gram negative spoilage microorganisms by extending lag phase and decreasing growth rate during log phase (Farber, 1991). To achieve the greatest extension of shelf-life, low temperature, close to 0°C (Farber, 1991; Gray et al., 1983), low initial microbial load (Lannelongue, 1982), and high concentrations of carbon dioxide (Blickstad and Molin, 1983) are necessary.

Shelf-life and keeping quality of raw channel catfish fillets have been reported to be 19 d for tray packed/chilled (2°C) products, 7 d for ice-packed fillets (Reed et al., 1983), up to 28 d under 80% CO₂ in barrier bags at 2°C (Silva and White, 1994), and about 16 d for fillets exposed to CO₂ for 6 d and transferred to trays wrapped with HDPE and held at 2°C (Silva et al., 1993). Initial counts on fish for those studies were 3.9 log CFU/cm², 3.9 log CFU/cm², 6 log CFU/g, and 5.8 log CFU/g, respectively. One MAP technology is the Master-Pack System. It consists of placing small product units (prepackaged or not) into a larger package units (Brody, 1989) which could then be evacuated and backflushed with a gas mixture. The products remain in this system until strict temperature control is not feasible or until ready for consumer display or use.

The objective of the present study was to compare storage life of channel catfish fillets held in different environments (air, vacuum and MAP - Master-Pack) and tray types (single or double-bed) at 2°C by measuring anaerobic, psychrotrophic, and Lactobacilli plate counts, pH, moisture and sensory scores (odor and appearance rating) every 4 d for 28 d.
MATERIALS AND METHODS

Materials

Live channel catfish *Ictalurus punctatus* were processed into fillets at a commercial catfish processing operation and packed in a walk-in cooler at 2°C in single-(S) or double-(D) bed Styrofoam trays with a moisture absorbant pad. The tray-packed fillets (2 per tray) were wrapped with a 2.7 mil polyethylene (HDPE) film and heat-sealed. The trays were packed in ice chests and covered with ice until further treatment at the Mississippi State University Food Science laboratories (not more than 6 h).

One third of the trays were stored as packed (AIR); one third were unwrapped and placed individually in permeable ETM bags (Cryovac Corp., Duncan, SC), vacuumized, and heat-sealed (VAC); and the other third were packed 5 trays to a bag in a barrier B700TM Master-Bag, air evacuated, and back flushed with CO2 to reach 90% CO2, 3% O2, and 7% N2 (MAP). The properties of each of the films are given in Table 1. All products were held at 2°C for up to 28 d and sampled periodically.

Each treatment was replicated twice and analyses were performed in duplicates. Treatments were tray-type (D or S), gas environment (AIR, VAC, or MAP), and storage time (up to 28 d) at 2°C.

Methodology

Anaerobic (AnPC), psychrotrophic (PPC) and lactobacillii (LAC) plate counts were performed following AOAC methods (FDA, 1992). Standard plate count agar (DIFCO Labs.) was used for AnPC (pour plates) and PPC (spread plates) with plates incubated at 25°C for 72 h in an anaerobic chamber and 21°C for 72 h in a temperature controlled chamber for AnPC and PPC, respectively. Rogosa agar (DIFCO Labs) was used for LAC, using pour plating technique and incubating aerobically at 25°C for 72 h.

Surface pH was measured using a flat-head electrode on the fish flesh as outlined by Silva and white (1994). Moisture was measured by shredding 5 g of fillet and heating at 100°C for 18 h (AOAC, 1990).

Appearance and odor ratings were conducted on the packaged products after sampling for microbial counts, by seven trained panelists. A 5-point rating scale for appearance and odor was used as follows: Appearance: 5 - fresh appearance 3 - slightly dry or slimy surface, 1 - slimy and off-colored surface; Odor: 5 - fresh, sweet odor, 3 - slightly spoiled (spoilage threshold rating), and 1 - totally spoiled/putrid.

RESULTS AND DISCUSSION

Initial anaerobic counts (AnPC) of fillets in double-(D) bed trays were lower than in single-(S) bed trays regardless of environment (Fig. 1). By the eighth day, AnPC were above 7 log CFU/g for all treatments except S/MAP, D/VAC, and D/MAP. By the 12 d, D/VAC products exceeded 7 log CFU/g, thus the product had spoiled (Handumrongkul and Silva, 1993; Martin and Hearnsberger, 1993; Anonymous, 1992). Products packed under 90% CO2 in single-bed trays (S/MAP) exceeded 7 log CFU/g after 16 d, whereas those packed in double-bed trays (D/MAP) did not exceed 7 log CFU/g until after 24 d.
Table 1. Properties of films used for packaging study.

<table>
<thead>
<tr>
<th>Film/Bag Type</th>
<th>Source</th>
<th>Thickness (mm)</th>
<th>WVTR (cm³/m²/24h/atm)</th>
<th>OTR</th>
<th>CO₂TR (cm³/m²/24h/atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>Dow Chem Co.</td>
<td>0.069</td>
<td>10.08 (0.65°C 100% RH)</td>
<td>1,070</td>
<td>3,350</td>
</tr>
<tr>
<td>E™ bag</td>
<td>Cryovac Corp.</td>
<td>---</td>
<td>-</td>
<td>4,000</td>
<td>16 - (22.8°C)</td>
</tr>
<tr>
<td>B700 bag</td>
<td>Cjovac Corp.</td>
<td>0.5-0.6</td>
<td>3-6</td>
<td>15-30</td>
<td></td>
</tr>
</tbody>
</table>

WVTR - water vapor transmission rate, OTR - oxygen transmission rate, CO₂TR - carbon dioxide transmission rate.
FIGURE 1. ANAEROBIC PLATE COUNTS IN CHANNEL CAT FISH FILLETS AS AFFECTED BY TRAY TYPE (SINGLE OR DOUBLE BED) AND ENVIRONMENT.
Psychrotrophic plate counts (PPC) were below 7 log CFU/g for single-bed or double-bed tray packed products in MAP (Fig. 2) for over 12 d. The PPC of fish packed under other treatments exceeded 7 log CFU/g after 4 d except for S/AIR which took about 6 d.

Lactobacilli counts (LAC) were lower in fillets packed in double-bed trays (Fig. 3), but reached the same levels as for other treatments after the 16th day, between 4-5 log CFU/g. It is well known that gram negative spoilage bacteria population is replaced by gram positive Lactobacillus in meat products under anaerobic or modified atmospheres (Farber, 1991; Gray et al., 1983).

Surface pH of fillets tended to be lower for products in double-bed trays for the first 12 d (Fig. 4). After the 16th day, pH of products packed under VAC or MAP in single-bed trays was near 5.0. This is probably due to carbonic acid and lactic acid formation (Brody, 1989).

Initial moisture content of fillets ranged from 72 to 81% (Fig. 5). Even though there was more visual drip in vacuum-packed products (data not shown), moisture content was not significantly affected. However, D/MAP fillets had lower moisture than S/MAP fillets after 28 d. This may be the result of the pad not being in direct contact with the fish in D trays.

Odor ratings of fillets in D/MAP and S/MAP were above spoilage level, 3 for at least 24 d (Fig. 6). Vacuum and air packed products were rated below 3 after 6 to 8 d at 2°C except for D/VAC, which were rated below 3 by the 12th day.

Appearance ratings of fillets followed closely the trends of odor ratings. Ratings for MAP fillets did not reach below 3 until the 28th day (Fig. 7), whereas the other treatments reached 3 between the 6th and 16th day.

Results show that fillets packed under 90% CO₂ (MAP) will have lower bacterial counts than those packed under vacuum or air. Lactobacillus counts show that spoilage bacteria are not rapidly replaced by Lactobacillus under MAP in double-bed trays but the latter grow rapidly in single-bed trays under MAP or VAC. The double-bed trays have a foam, porous layer between the product and the pad, thus isolating the product from the drip, and the bacterial flora accumulated away from the product. Lower pH is thought to be from the conversion of CO₂ to carbonic acid and the production of lactic acid by lactobacilli (Daniels et al., 1985; Lannelongue et al., 1982). Odor (Fig. 6) and appearance (Fig. 7) ratings of fillets followed anaerobic (Fig. 1) counts (AnPC) very closely, and correlated somewhat with PPC (Fig. 2). A slimy, whitish appearance of the fish surface and an ammonia, putrid smell were the signs of fish spoilage, although MAP products also had a sour smell towards the end of their storage (24-28 d).

This work shows that not only products packed under 90% CO₂ in a Master-Bag will have a longer shelf-life, but also products packed in double-bed trays may have longer keeping quality. Also it shows that AnPC may follow better the microflora of fish fillets held at 2°C as compared to PPC.
FIGURE 2. PSYCHROTROPHIC PLATE COUNTS IN CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
FIGURE 3, LACTOBACILLI PLATE COUNTS IN CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
FIGURE 4. CHANGES IN pH OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
FIGURE 5, CHANGES IN MOISTURE CONTENT OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
FIGURE 6. ODOR RATING OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
FIGURE 7. APPEARANCE RATING OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.
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REFERENCES


