INTRODUCTION

The purpose of the paper is to examine the characteristics and significance of disease associated with the bacteria *Vibrio vulnificus* in oysters and how this affects public health policy. It is necessary that we determine the nature of the organism, describe the disease with which it is associated, determine its environmental distribution, determine the public health risk, discuss the state’s legal liability as well as moral and ethical obligations, and outline the proposed responses and their potential impacts upon the public and the industry.

By understanding the nature of the organism we can understand the implications of causing disease via oysters. Oysters filter the water in their environment to extract their food. *Vibrio vulnificus* (V. *vulnificus*) is a marine bacterium which is found throughout the marine environment within U. S. coastal waters (9, 10, 14, 19, 21, 22). It is also found in brackish water environments (13). The bacteria are highly motile, free living, gram negative, curved, rod-shaped, autochthonous inhabitants of the waters from which molluscan shellfish are harvested (5, 16). *V. vulnificus* is a halophilic, (salt-loving) non cholera, marine vibrio which is most frequently isolated from marine environments with a temperature greater than 20°C (68°F) and a salinity of 7‰ to 16‰ and is only rarely found in environments cooler than 17°C (62.6°F) (20). Oysters harvested from waters which exhibit these salinity and temperature regimes have the potential to cause *V. vulnificus* infections. Since *V. vulnificus* occurs in Virginia waters which exhibit these specific characteristics, we must address the disease caused by this infection.
Description of the organism

In order to perceive the public health implications of the presence of V. *vulnificus* in oysters, we must understand how the infection can occur, what are its manifestations and what is the prevalence rate of the disease. *V. vulnificus* is a highly virulent and invasive pathogen which is identified with three different disease syndromes (9, 23). The first syndrome is a progressive infection characterized by a few diarrheal symptoms, primary septicemia and cutaneous lesions, it carries a 50% case fatality rate (7). The second is a wound infection which results in skin lesions and occasionally septicemia, with a 13% case fatality rate (4, 13, 23). The third is acute diarrhea which is limited and rarely fatal (12). Infection occurs most often in immunocompromised hosts which include patients who have neoplasia of the immune system, a hematopoietic disorder, liver disease or alcoholism, chronic renal failure, or acquired immune-deficiency syndrome, or patients who are receiving pharmacological immunosuppression for neoplasia or transplantation of an organ (8). Additionally, patients with diabetes mellitus, individuals with naturally low levels of gastric acid or those taking prescription or over-the-counter medication to reduce stomach acidity are at increased risk of infection (12). Those persons with hepatic disorders and iron metabolism dysfunction are at much higher risk of progressive infection which often results in death (14, 18). Infection with *V. vulnificus* comes from exposure of lesions to the marine environment, injuries from marine animals, or the ingestion of raw or partially cooked shellfish (13, 14, 18).

Epidemiology

In Virginia the incidence of *V. vulnificus* infections has been low in comparison to the Gulf states. From 1974 to 1992 there were 29 cases of *V. vulnificus* infections identified by the Virginia Division of Consolidated Laboratories and reported to the office of Epidemiology; 3 resulted in death (VDH Epidemiology program 1992). The average annual incidence for this period in Virginia is 0.53 cases per 100,000 population with a mortality rate of 0.05 deaths per 100,000.

In the state of Florida from 1981 through 1992 there were 1,124 confirmed cases of *V. vulnificus* infections; 43 resulted in death (Florida Department of Health and Rehabilitative Services Epidemiology Program 1992). The average annual incidence for this period in Florida is 1.09 cases per 100,000 population, with a mortality rate of 0.37 deaths per 100,000.

In the state of Louisiana there were 85 confirmed cases of *V. vulnificus* infections from 1981 through 1992, of which 25 resulted in death (Louisiana Department of Health and Hospitals, Epidemiology program 1993). The average annual incidence in Louisiana is 2.01 cases per 100,000 population, with a mortality rate of 0.59 deaths per 100,000.

The demonstrated incidence of *V. vulnificus* infections in a Maryland Chesapeake Bay community was 1.6 per 100,000 per year from 1974 through 1988 (6). These numbers indicate a significantly higher incidence rate demonstrated in this clinical study than the reported Virginia annual incidence rate. These clinical studies tend to provide more comprehensive data than the state reports. The state statistics may not be accurate due to underreporting, improper diagnosis and other confounding factors. Therefore, the average annual incidence rate in Virginia may be higher than 0.53 cases per 100,000 population.
Environmental Distribution

The next step is to uncover why the incidence rates are higher in the Gulf than in Virginia. The key to answering this question is found in determining how the organism is distributed in the environment. Concentrations of *V. vulnificus* vary widely in the water column sediment and oysters. The recent cooperative work between the Virginia Department of Health, Division of Shellfish Sanitation, and the University of Florida has shown that there is a statistically significant difference between Gulf of Mexico waters and Chesapeake Bay waters. The concentrations of *V. vulnificus* are, on average, somewhat greater in the Gulf waters and persist for longer periods presumably due to higher temperatures, a more temperate yearly climate and higher salinities.

A number of studies indicate that there is a strong correlation between temperature, salinity, and the presence of *V. vulnificus* in seawater and oysters (9, 10, 11, 14, 15, 17) As stated earlier *V. vulnificus* has been isolated from waters with a wide range of temperature and salinity readings, but the highest concentrations of the organism have been found in waters with temperatures of 17°C to 31°C and salinities of 15‰ to 25‰ (17).

Most oysters harvested in Virginia come from waters that fall outside the temperature and salinity ranges preferred by *V. vulnificus*. The majority of oysters presently harvested in Virginia are from waters with salinities less than 15‰. Harvesting on public oyster grounds is limited to the months from October to May. The oyster harvest statistics from the Virginia Marine Resources Commission indicate that the months, in order of number of bushels of oysters caught, for the years 1988 to 1992 are January, October, November, December, February, March respectively (Virginia Marine Resources Commission, Fisheries Plans and Statistics, 1993). The average monthly water temperatures (calculated from the daily mean) for the months October to May, from 1947 to 1992, for the Chesapeake Bay watershed (as measured at the Virginia Institute of Marine Science pier) range from a low of 4.12°C in January to a high of 26.61°C in August with the months of November through April having average temperatures below 17°C (Virginia Institute of Marine Science, 1993). Therefore, it is less likely that oysters from Virginia will have concentrations of *V. vulnificus* as great as those from the Gulf states. Additionally, the majority of oyster landings in Virginia occur in months when the water salinities are outside of the optimum range for the growth of *V. vulnificus*. Consequently, the likelihood of oysters harvested from Virginia having significant numbers of *V. vulnificus* is low whereas for the Gulf oyster it is high. We must address the risks associated with oysters caught from Virginia and those caught from the Gulf.

Public Health Risks

What are the public health risks posed by *V. vulnificus* in oysters that are harvested, distributed and processed in Virginia? In order to adequately address this question we must outline how the oyster industry in Virginia currently operates. The season of oyster harvesting from areas open to harvest by anyone who purchases the appropriate licenses is October through May. Harvest from private leases from the state occurs year round. The major portion of the processing takes place from October through March. Due to the shortage of Chesapeake Bay oysters, their prices are high. In contrast, due to their abundance, Gulf oysters are, at times, only half the price of Virginia oysters. So, based on price differential and availability of the resource, oysters from other states, particularly
Gulf states, are imported into Virginia to be processed. The oysters that come to Virginia from Florida, Louisiana and Texas have a warning label. After these oysters are processed, they go out under the label of a Virginia facility. Since Virginia does not require a warning label, the information concerning the risks associated with these oysters is lost.

Risk is a quantitative assessment of the degree of harm to health that may be anticipated. Safety is a value judgement of how much risk an individual or a community is willing to accept (2). These concepts are crucial in our examination of this issue.

As noted earlier, the disease and death rates from *V. vulnificus* infections are much lower in Virginia than in Florida and Louisiana. The results of the 1993 Commonwealth Poll estimate that there are 847,554 raw shellfish consumers in the state of Virginia (18.1% of the population 18 or older). Of this group, 92,882 (10.9%) have at least one high risk characteristic. These high risk characteristics are liver disease, stomach disorders, diabetes, immune system disorder or immune suppressant drug therapy. The 1988 Florida Behavioral Risk Factor Survey estimated the prevalence of raw oyster consumption and host factors that may facilitate *V. vulnificus* infections (4). Approximately 2,950,818 adults in Florida are raw oyster eaters (23% of the population 18 or older) and 70,820 (0.5%) of these raw oyster eaters have liver disease (one of the high risk characteristic) (3).

There appears to be a lower risk of disease and death from eating oysters caught in Virginia. Some of the possible explanations are: 1) The concentration of *v. vulnificus* in Virginia oysters is lower. 2) The majority of oysters are harvested in the months where the water temperature is lower and, therefore, there are fewer organisms in the water and in the oyster. 3) The salinities in the areas where oysters are harvested do not provide optimum conditions for the proliferation of the organism. 4) Consumers are educated and ask for oysters caught in Virginia. 5) The serotype of *V. vulnificus* found in the Gulf is more virulent that of the serotype found in Virginia waters.

Estimates indicate that approximately 90% of the oysters processed in Virginia are from the Gulf. The greater risk of disease and death from eating oysters caught in Gulf waters may be explained as follows: 1) The concentrations of *V. vulnificus* in Gulf oysters is higher than Virginia oysters. 2) During the months in which the public oyster harvest season in Virginia is closed, processors may purchase oysters from the Gulf. It is during these summer months that the water temperatures are highest and, therefore, the concentrations of *V. vulnificus* would be highest. 3) Salinities in the areas of harvest in the Gulf provide the optimum conditions for the proliferation of the organism. 4) Shucked oysters are not identified as to the place of harvest, so consumers are not aware that they may be buying Gulf oysters.

Florida has shown through their work that there is a substantial difference in the risk of disease and death between nonraw oyster eaters, raw oyster eaters and raw oyster eaters with liver disease. Of the oysters implicated in *V. vulnificus* infections in Florida all were harvested from the waters of the Gulf or southern Florida. (Fla. HRS Epidemiology Program)
RELATIVE RISK RATIOS FROM FLORIDA DATA

NONRAW OYSTER EATERS
.02 illnesses/l 000 adults/35 years; .003 deaths/1000 adults/35 years

RAW OYSTER EATERS
.03 illnesses/l000 adults/35 years; .008 deaths/l 000 adults/35 years

RAW OYSTER EATERS WITH LIVER DISEASE
2.43 illnesses/l000 adults/35 years; 1.53 deaths/l 000 adults/35 years

There is also a risk of *V. vulnificus* infection via a wound exposed to the marine environment. This risk exists obviously for anyone exposed and increases if they have a liver disorder, are immunocompromised or exhibit any other factor which may increase their susceptibility. The mortality rate for a wound infection is significantly smaller than for primary septicemia.

Legal Liability

If someone is infected with *V. vulnificus* from oyster ingestion and dies, the state’s legal liability will be questioned. This issue has arisen in California and Louisiana.

In a recent California case, an individual became sick from ingesting raw oysters from the Holiday Inn at Fisherman’s Wharf in San Francisco (Kilpatrick v. Holiday Inns Inc.) The attending physician concluded that the symptoms resulted from the consumption of raw oysters tainted with *Vibrio cholerae*. The man sued the Holiday Inn and the companies (United Shellfish, Inc., Fisherman’s Wharf Seafoods, Inc. and Pearson’s Seafood) that supplied and transported the shellfish. The suit was based upon causes of action for negligence, negligence per se, strict liability and breach of warranty. This case was settled out of court with the trucking companies that shipped the shellfish paying damages to the plaintiff.

In Louisiana a Mississippi man became infected with *V. vulnificus* from consumption of Louisiana oysters (William Winstead v. Ed’s Live Catfish & Seafood Inc., et al. Parish of E. Baton Rouge, LA) (17). The original verdict found that the state was liable for not having adequately warned the public of the risks associated with consuming raw oysters. The case was appealed and the lower court’s ruling was overturned. The most important findings are as follows:

1. The failure of the Department of Health and Human Resources to warn the consumer that a person with certain underlying risk factors should not consume raw oysters was not a cause, in fact, of consumer’s illness. The Department was not liable since the consumer was not aware that he had any of the underlying risk factors.

2. Since the Department of Health and Human Resources had undertaken studies of *Vibrio vulnificus* bacteria and knew that it posed risk to a small segment of the population, it had a duty, once it had sufficient information, to issue a warning to the public concerning the health hazards possibly associated with *Vibrio vulnificus*.
bacteria in raw oysters. However, the Department fulfilled its duty by publishing a report summarizing what the Department knew about bacteria at the time and by stating that physicians should warn patients with any of the underlying risk factors not to eat raw oysters.

3. It was an error for the trial court to create a duty on the DHHR to warn when no such duty exists in Louisiana statutory or case law.

4. It was an error for the trial court to conclude any act or omission of DHHR was a cause, in fact, of the plaintiff’s injury.

5. It was an error for the trial court to conclude that the DHHR did not warn the public about the Vibrio vulnificus bacteria and its relationship to raw oyster consumption.

A similar case was also brought to court in Louisiana (Theresa Simeon, et al. v. Big Easy, Inc., M.J. Billick Oyster Co., Dept. of Wildlife and Fisheries, La. Dept of Health Civil District Court New Orleans, LA)( 18). In this case Floyd Simeon Sr. and his son ate raw oysters at the Sweet Pepper grill in New Orleans. Thereafter, Simeon was hospitalized for pain associated with severe blisters on his legs. After attempts to stop the spreading of the blisters failed, Simeon’s legs were amputated. Despite these efforts, Simeon subsequently died. Death resulted from septicemic infection caused by V. vulnificus. Mr. Simeon’s daughter later brought suit against the restaurant, suppliers and the Louisiana state agencies that hold responsibility for monitoring the sanitation of oysters. As with the previous case, the original verdict found that the state was liable for not having adequately warned the public of the risks associated with consuming raw oysters. The case was appealed and the lower court’s ruling was overturned. The most important findings are as follows:

1. The Department of Health and Human Resources (DHHR) fulfilled its duty to warn concerning danger of bacteria in raw oysters by publishing monthly morbidity reports targeting the warning to health care providers. Since the bacteria most often attack a small percentage of the population, ie. those with liver disease, kidney disease, or other immune-suppressive condition, this method of warning was determined to be adequate.

2. A newspaper article quoting Department of Health and Human Resources (DHHR) officials concerning dangerous bacteria present in raw oysters was offered to show that the public was warned that the dangers in eating raw oysters are relevant, not hearsay.

3. Statistics from United States Department of Commerce, National Marine and Fisheries Service, offered to prove extremely low incidence of bacteria infection in oysters and to show it was more prudent to target warnings to health care providers, who would inform those very few who were at risk, were determined to be admissible in action arising from the death of restaurant patron.
4. The fact that the DHHR chose not to aggressively disseminate the information contained in the Monthly Morbidity Report to the general public does not constitute a breach of the duty to warn. Since the bacteria only attacks a small percentage of the population, i.e., those with liver disease, kidney disease or other immune-suppressive condition, the DHHR was reasonable in its conclusion that the best way to warn these people was through the medical community. The Monthly Morbidity Report was sent to every physician in Louisiana and the health departments of several states, including Mississippi. The court held that this satisfied the duty to warn which was incumbent on the DHHR due to its specialized knowledge and position of public trust.

In this case one judge on the Fourth Circuit Court of Appeals of Louisiana held a dissenting opinion. In this minority opinion he writes: “Considering the DHHR’s knowledge about the extreme danger of this bacteria and its position of public trust, I find that DHHR owed a duty to the general public to adequately warn of the possibility of contracting a deadly disease by eating raw oysters. A warning to the medical community absent a concurrent warning to suppliers and retailers of oysters as well as the general public was not sufficient.”

The liability of the state as determined by the court indicates that, if the effort is made to inform and educate about the risks associated with raw oyster consumption, then state liability is minimized. This effort is considered valid if it is targeted toward the medical community and the general public via the media. Warning labels on the product are not deemed to be necessary in order to fulfill the agencies’ responsibility to warn.

Proposed Response To The Issue

There is an increased risk of disease from eating raw oysters. Due to the ubiquitous nature of many bacteria and their opportunistic abilities, they will always pose a risk to those who eat food which has not been subjected to a bactericidal process. There currently is no feasible method available in harvesting, processing or distribution to render the oyster free of harmful bacteria without cooking it. Therefore, disease prevention must focus on educating those at the greatest risk (ie. raw oyster eaters with liver disease, stomach disorders, and immune deficiency disorders) about the magnitude of risk associated with this behavior.

The Interstate Shellfish Sanitation Conference (ISSC) is a consortium of state agencies, federal agencies and industry that formulates policy for adoption by the United States Food and Drug Administration (FDA) into the National Shellfish Sanitation Program (NSSP). It is this program that provides the specific guidelines of sanitation which must be followed by the industry and the operational rules under which the states must implement their programs. The purpose of the NSSP is to provide uniformity and to assure that shellfish shipped interstate are safe. The ISSC has addressed the V. vulnificus concerns as follows:

1. In Issue 92-311, the ISSC adopted: “The Interstate Shellfish Sanitation Conference should formally address the public health significance of naturally occurring pathogens and biotoxins associated with the consumption of raw molluscan shellfish. Specifically, the ISSC should continue to investigate and address harvest, handling,
storage, process, distribution, education, and public information activities particularly those activities targeted toward those at-risk consumers.”

2. In Issue 92-117, the ISSC adopted: “ISSC should seek technical assistance from federal agencies regarding water temperature and naturally occurring pathogen levels in water and shellstock at different storage temperatures and its public health significance. In addition, an evaluation of commercial feasibility should be conducted.

3. In Issue 92-304, the ISSC adopted: “The Interstate Shellfish Sanitation Conference should work with FDA and industry through the Education Committee to develop educational programs to reduce health risks associated with consuming raw shellfish. Such programs should be geared to both the general public and at-risk groups. The Conference should develop consumer education brochures targeted to at-risk groups and the medical/health community, promote Dear Doctor’ letters to physicians, and develop other methods of educating consumers.”

4. In Issue 92305 the ISSC adopted: “...as part of the ISSC on-going educational efforts, if individual States require a point-of-sale message, the following language is recommended:

CONSUMER INFORMATION

As is the case with consuming other raw protein products, there is a risk associated with consuming raw shellfish. If you suffer from chronic illness of the liver, stomach or blood or have immune disorders, do not eat these products raw.”

The position of the ISSC is that: “Certain individuals with underlying medical problems (cirrhotics, diabetics, persons with renal failure, persons with hemochromatosis, persons who are immunosuppressed) are at high risk for infection by Vibrio vulnificus. The Conference has stated and continues to emphasize that such individuals must not consume raw molluscan shellfish. The Conference believes that aggressively targeted educational efforts are appropriate means of reducing illnesses from Vibrio vulnificus. Such targeted educational efforts are appropriate means of reducing risk population through their various support groups (i.e. professional, medical, social, voluntary, and governmental organizations). Further aggressive Conference actions include recommendations to states and the shellfish industry to take immediate steps to institute harvesting, processing, and handling procedures which will eliminate illnesses attributable to Vibrio vulnificus. These procedures should include appropriate rapid cool-down and continued chilling of shellfish to control growth of potentially pathogenic Vibrio. In addition, the ISSC. In addition, the ISSC will promote and encourage research to develop control mechanisms which will effectively reduce these illnesses.” (ISSC News Release, March 21, 1991).
The position of the FDA is that: “Reports of illness and death attributable to Vibrio vulnificus infection following the ingestion of raw oysters emphasize the importance of warning certain high-risk patients against eating raw or lightly (partially) cooked seafood. These patients include those with:
- liver disease and other diseases with possible or definite hepatic involvement, such as alcoholism, malignancy, hemochromatosis, thalassemia major,
- therapeutically induced or naturally low gastric acid,
- compromised, immune systems (including AIDS and AIDS-related complex). There are presently no known sanitation or other public health controls that can limit the harvesting of shellfish to those free of *Vibrio* or control the presence of the organism in raw shellfish in the marketplace. *Vibrios* are not expected to withstand normal cooking procedures. Shellfish-borne *Vibrio* infections can be prevented by cooking seafoods thoroughly and keeping them from cross-contamination after cooking. Cooked seafood is best stored at 60 degrees C (140 degrees F) or greater, or 4 degrees C (39.2 degrees F) or below. *Vibrio vulnificus* is naturally occurring in the marine environment, and it is considered to be an opportunistic pathogen in individuals having compromised immune systems or medical disorders. The control measure of choice at this time is adherence to Good Manufacturing Practices associated with shellstock handling and transportation.” (FDA Drug Bulletin, April 1988, Volume 18, Number 1).

The FDA has agreed with the ISSC “Issues” 92-311, 92-304, and 92-305 (October 9, 1993 letter from FDA to the ISSC).

Both FDA and the ISSC recommend that this issue be investigated and addressed by analyzing and examining harvest, handling, storage, process, and distribution of the product. This recommendation is currently being pursued by the ISSC and the VDH. Additionally education and public information activities, particularly those activities targeted toward at-risk consumers, should be initiated. The FDA and ISSC feel that consumer education brochures should be targeted toward at-risk groups and the medical/health community by promoting “Dear Doctor” letters to physicians, and by developing other methods of educating consumers.

**CONCLUSION**

*Educating* persons who have liver disease, stomach disorders, or immune deficiency disorders about the risk in eating raw oysters is most effectively accomplished via the physician because 1) the physician has the most complete knowledge of the patient’s state of health and therefore, can assess individual risk, 2) the patient is more likely to heed a physician’s advice as opposed to a general warning via a label, notice in eating establishments, news releases etc.; and 3) one-on-one communications are more effective than mass communications.

An informed physician can be the most effective communicator of the risks associated with raw oyster consumption to high risk patients. If we target specific physicians, we have a smaller, more informed audience to reach with the information and can more easily repeat the message so that it is reinforced. If we wish follow up to assure that the message is getting to the physicians and that they are relaying it to their high risk patients, we can measure the effectiveness of our effort.
Optimally, the information should be available in a pamphlet form in the physician’s waiting room as a mechanism to reach more individuals. The groups that are at highest risk of infection from eating raw oysters are more likely to be visiting a physician. By placing this information in the waiting room area, the individual may be stimulated to question the physician concerning consumption of raw oysters. In addition to educating the physician, this would increase the likelihood that the consumer at greatest risk would be informed and that the physician would provide advice on this matter.

The appropriate agency should also distribute a news release concerning the risks involved with the consumption of raw oysters. This release could be provided on a yearly basis to reinforce the message. It would also be sent to all other state public health agencies so that they may pass this information on to their citizens as they see fit.

Warning labels on the product are another alternative but warning labels tend not to elicit the positive response that physician’s advice would. According to Dr. Louise McFarland, the director of the Epidemiology program in Louisiana, warning labels on the product are not entirely effective. That state has not seen a significant decrease in cases of disease or death as a result of its warning label program. She said that a well, directed public education campaign which included health professionals was a much more effective method of reducing risk than warning labels. Warning labels may not be taken seriously. In most instances, warning labels rarely reach the intended audience because, if they are not included at the point of sale (ie. usually at the restaurant or retail outlet), the information does not get to the consumer. Indications from states that have labeling requirements are that they actually have little effect on reducing the number of cases of *V. vulnificus* infections via oyster consumption. The majority of health professionals that we have surveyed indicate that, in their opinion, warning labels are the least effective means by which to educate the public concerning the risk of eating raw oysters and that information disseminated to health professionals and the mass media was most effective. We share this opinion and recommend a comprehensive public education/information campaign be implemented in Virginia.

In order to implement the physician and general public education campaign, it will be necessary to communicate the message via mailings, distribution of printed information at physicians’ organization meetings, speaking at physician meetings, information printed in professional journals, provision of printed materials to be placed in physicians’ office waiting rooms, and through news releases to both the print and electronic media. It will be necessary to follow up with a mail or telephone survey to estimate the effectiveness of this campaign.

There are certainly some other more controversial measures that can be taken to minimize the potential risk of disease. These include restricting harvesting from Gulf waters to months which exhibit lower concentrations of *V. vulnificus*, require an approved irradiation regimen for all shellfish, require thermal processing of all shellfish prior to marketing and restrict the sale of raw shellfish. Are these measures reasonable in light of the potential risk? Probably not, but they will certainly continue to be proposed as long humans continue to contract vibriosis from ingesting raw shellfish. An educated public is the best line of defense against this disease.
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PROPERTIES OF FISH GELATIN

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During the manufacture of gelatin, collagen from connective tissue (skin, bone, sinew) is converted into a soluble, gel forming material which can be used as a food component or as a glue. Gelatin, commonly processed from pork and beef, is widely used as a commodity because of its gel forming and surface active properties. However, the Kosher/Halal community does not accept most currently available gelatin because the processing does not meet their religious requirements. Although a few low Bloom (less than 100 Bloom) fish gelatins have been available to this community, the gelatins were not comparable to commercial high Bloom gelatins. In order to fill this need, a few companies, including Food Industry Testing, Inc. (Miami Beach, FL), have developed a process (currently being patented) to obtain a high Bloom fish gelatin from warm-water fish skins. This product, in addition to the great benefit it represents to the Kosher/Halal community, has a high value to the fish industry’s waste management effort by yielding an additional income for a by-product that is commonly wasted. The two main purposes of this study were to study the properties of these high Bloom fish gelatins derived from fish skin and to compare them to the ones from a well-known commercial gelatin.

MATERIALS AND METHODS

Seven fish gelatin samples: 300 Bloom fish gelatin (lots T5001, T5401, TA30104, and T5104), 250 Bloom fish gelatin (lot T25104), 210 Bloom fish gelatin (lot T5113), and 200 Bloom fish gelatin (lot T5502), were investigated. In addition, a commercial product, Knox® gelatin (Englewood Cliffs, NJ, lot 5-35001-08) was studied alone, and mixed 25%
50% and 75% by weight with 300 Bloom fish gelatin (T5001) or with 200 Bloom fish gelatin (T5502). All these fish gelatin samples were prepared into 5% (w/w) solutions for the study of the effect of dissolving time on solubility, pH, melting point, and minimum solubility. For the determination of the minimum solubility point and the effect of concentration on pH, melting point and gel strength, the 300 Bloom fish gelatin (T5104) was used.

**Effect of dissolving time on fish gelatin solubility**

Fish gelatins (300 Bloom (T5104) and 200 Bloom (T5502)) solutions were prepared with deionized distilled water and kept at 40°C for 0, 1, 2, 3, 4, 5, and 6 hr. Each sample was centrifuged at 16,000 rpm for 15 min at approximately 40°C and the protein content of the supernatant was determined by Lowry (1951). The solubility was expressed as the percent of total protein (measured prior to centrifugation).

**pH in solution**

pH was measured using a glass electrode pH meter (pH-103 Metrohm/Brinkmann, Brinkmann Instruments Inc., Westbury, NY). All solutions were prepared with deionized distilled water and equilibrated to 40°C in a water bath for at least one hour. The calibration buffer (Certified Buffer Solutions, pH 4.00±0.01 @ 25°C, Fisher Scientific Co., Fail Lawn, NJ, lot 87623-24) was also kept in the water bath.

**Minimum solubility**

Eight 30 mL fractions of the 5% solution were prepared and kept at 40°C for at least one hr. The pH was adjusted with 0.1N HCl or 0.1N NaOH to pH 3.8 to 5.2 (0.2 pH increments). These samples were centrifuged at 16,000 rpm for 15 min at approximately 40°C. A Lowry protein determination of the supernatant gave the total soluble protein (Lowry, 1951). The pH of the fraction with the lowest protein content in the supernatant (i.e., less soluble protein) was defined as the "minimum solubility point." After the approximate minimum solubility point was determined, the pH range was reexamined using 0.1 pH increments. The minimum solubility was calculated as a percent of the total sample weight and as a percent of the total protein (measured prior to centrifugation).

**Melting point**

The melting point measurement method described by Wainewright (1977) was modified. The test determines the temperature at which gels soften sufficiently to allow drops of chloroform to sink through them. Solutions were prepared with 0.1N NaCl, equilibrated at 40°C, and the pH adjusted to 7.0 with 0.1N NaOH. A 5 mL aliquot of each sample was transferred to a small glass tube (Fisherbrand® borosilicate disposable culture tubes, 12 x 75 mm, Fisher Scientific Co., Pittsburgh, PA) previously coated with Sigmacote® (Sigma Chemicals Co., St. Louis, MO, lot 23H6097) to prevent sticking. The samples were degassed in a vacuum desiccator for 5 min. The tubes were then covered with Parafilm® (Laboratory Film, Greenwhich, CT). After heating in a
water bath at 90°C for 15 min, the gelatin solutions in the glass tubes were cooled immediately in ice-chilled water to 10°C and matured for 4 hr. Five drops of a mixture of chloroform and a reddish brown dye (Food color AFO OWS 550, Miles Inc., Elkhart, IN, lot 5-057039) were placed on the surface of the gel. The gels were put in a water bath at 10°C and the bath was heated at 0.2-0.4°C/min. The temperature of the bath was read from an electronic digital thermometer (±0.2°C, NBS (NIST), Serial No. 457261). The temperature at which the chloroform drops began to freely move down through the gel was taken as the melting point.

**Gel strength**

This method was modified from the NE-123 (Northeast Regional Hatch Protein Functionality Group) gelation method (Chong Lee, personal communication). Solutions from 2% to 12% (w/w) were prepared with 0.1N NaCl and equilibrated to 40°C. The pH was adjusted to 7.0 with 1N NaOH and the solutions stirred for 30 min. Following hydration, the samples were transferred to glass tubes (19 x 175 mm) which were previously coated with Sigmacote® and closed on one end with rubber stoppers (Fisherbrand® No. 2, Fisher Scientific Co., Pittsburgh, PA). The samples were degassed in a vacuum desiccator for 10 min. The other end of each glass tube was closed with a second rubber stopper, to prevent moisture loss and cook swelling, and the glass tubes were placed vertically in a 90°C water bath for 15 min. The glass tubes were then cooled to 10°C immediately in ice-chilled water for 20 min and kept refrigerated (4-10°C) overnight. The gels were removed from the glass tubes and cut into about 8 cylinders 19 mm long. Each cylinder was then compressed on an Instron Universal Testing Machine (Canton, MA, Model No. 1101) to determine the failure force. The cross-head (CCT load cell, 200 lb) speed of the Instron, interfaced with an IBM PC-XT, was set at 12.7 cm/min. The gel strength of each sample was calculated from the average failure force of several cylinders.

**Sensory analysis**

Cubes with 2.75% gelatin (w/w) were made from 300 Bloom (T5104 and TA30104) or 250 Bloom fish gelatin and Knox® gelatin, and cranberry juice (Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA) or grape juice (Sunny Square grape juice, Commander Foods, Inc., Syracuse, NY). One third of the juice was added cold to the fish gelatin and allowed to sit for 1 min. The rest of the juice was heated (high) in a microwave (Amana Touchmatic II Radarange® microwave oven, Amana Refrigeration, Inc., Amana, IW) for 1 minute, added to the cold mixture, and stirred until the gelatin was completely dissolved (about 5 min). Because the color of the fish gelatin was different from the Knox®, a few drops of red food color (McCormick, Hunt Valley, MD) was added. The mixture was poured into a 9” square pan and kept overnight in a refrigerator, and then cut into 1” cubes. A triangle sensory test (Larmond, 1987) was used to determine if the panelist could tell the difference
between the two products made from fish gelatin and Knox®. The panelists were also asked to record how great the difference was and which sample(s) (odd or pair) was preferred. Only correct answers on the triangle test were kept for further evaluation.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of dissolving time (i.e., the time between gel solution preparation and the solubility test) on the protein solubility (%). The major change (i.e., a difference in solubility of about 12%) seemed to occur during the first hr. After 1 hr, the solubility changed slightly (i.e., an increase in solubility of about 3%) for both samples. Based on these results, all the samples that were prepared for the rest of the tests were allowed to stand for at least 1 hr.

Table 1 shows pH, melting point, the minimum solubility point, and the minimum solubility of all samples. The pH ranged from 4.98 to 5.27. The lowest pHs were observed in 210 Bloom (T5113) and 300 Bloom (T5104) gelatin. The rest ranged from 5.17 to 5.27. The pH values were found to be significantly different by one way ANOVA statistical analysis from Bloom to Bloom and even within a single Bloom (300) (p<0.05). However, the pH range was only of 0.29 pH units. The pH of the mixtures of 300 Bloom and Knox® were not significantly different from either 300 Bloom or Knox®. On the other hand, the pH of the mixtures of
200 Bloom and Knox® were significantly different from Knox® ($p<0.05$) but not from 200 Bloom fish gelatin.

### Table 1. pH, melting point, minimum solubility point (MSP) and minimum solubility (MS) of gelatins as 5% (w/w) solutions

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH</th>
<th>Melting Point (°C)</th>
<th>MSP (pH)</th>
<th>MS (% of Total Protein)</th>
<th>MS (% of Total Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 Bloom fish gel (T5001)</td>
<td>5.21</td>
<td>24.3</td>
<td>4.34</td>
<td>97.29</td>
<td>83.03</td>
</tr>
<tr>
<td>300 Bloom fish gel (T5401)</td>
<td>5.22</td>
<td>24.7</td>
<td>4.35</td>
<td>96.89</td>
<td>82.13</td>
</tr>
<tr>
<td>300 Bloom fish gel (TA30104)</td>
<td>5.27</td>
<td>24.7</td>
<td>4.38</td>
<td>96.33</td>
<td>84.19</td>
</tr>
<tr>
<td>300 Bloom fish gel (T5104)</td>
<td>5.05</td>
<td>24.6</td>
<td>4.38</td>
<td>96.66</td>
<td>84.08</td>
</tr>
<tr>
<td>250 Bloom fish gel (T25104)</td>
<td>5.27</td>
<td>24.0</td>
<td>4.38</td>
<td>95.95</td>
<td>86.09</td>
</tr>
<tr>
<td>210 Bloom fish gel (T15113)</td>
<td>4.98</td>
<td>23.7</td>
<td>4.38</td>
<td>96.24</td>
<td>84.93</td>
</tr>
<tr>
<td>200 Bloom fish gel (T5502)</td>
<td>5.20</td>
<td>23.5</td>
<td>4.38</td>
<td>95.42</td>
<td>85.55</td>
</tr>
<tr>
<td>Knox® Gelatin</td>
<td>5.17</td>
<td>29.6</td>
<td>4.34</td>
<td>96.34</td>
<td>83.41</td>
</tr>
<tr>
<td>25% 300 Bloom fish gel (T5001) + 75% Knox®</td>
<td>5.20</td>
<td>28.6</td>
<td>4.35</td>
<td>96.71</td>
<td>83.07</td>
</tr>
<tr>
<td>50% 300 Bloom fish gel (T5001) + 50% Knox®</td>
<td>5.18</td>
<td>27.3</td>
<td>4.35</td>
<td>96.19</td>
<td>82.74</td>
</tr>
<tr>
<td>75% 300 Bloom fish gel (T5001) + 25% Knox®</td>
<td>5.19</td>
<td>25.9</td>
<td>4.36</td>
<td>96.32</td>
<td>82.89</td>
</tr>
<tr>
<td>25% 200 Bloom fish gel (T5502) + 75% Knox®</td>
<td>5.21</td>
<td>26.8</td>
<td>4.35</td>
<td>95.47</td>
<td>83.74</td>
</tr>
<tr>
<td>50% 200 Bloom fish gel (T5502) + 50% Knox®</td>
<td>5.24</td>
<td>26.3</td>
<td>4.35</td>
<td>95.55</td>
<td>84.23</td>
</tr>
<tr>
<td>75% 200 Bloom fish gel (T5502)+25%Knox®</td>
<td>5.23</td>
<td>25.2</td>
<td>4.36</td>
<td>95.16</td>
<td>84.72</td>
</tr>
</tbody>
</table>

The concentration of the solution seemed to affect the pH slightly (Fig. 2), although from 2% to 20% gelatin the shift was only about 0.10 units.
Two tests were performed to measure the minimum solubility of 5% 300 Bloom (T5104) (Fig. 3). Since the lowest solubility was observed at pH 4.38 in both cases, this value was considered the minimum solubility point of this fish gelatin sample. The minimum solubility point of all samples (see Table I) showed a mean of 4.36±0.02 pH units. The minimum solubility was 96.18±0.02% of the total protein content or 83.91±0.13% of the total weight. Furthermore, all 200 Bloom to 300 Bloom samples had a minimum solubility of about 96.14±1.39% of total protein content which was very close to the minimum solubility of Knox® of about 96.34% of total protein content. This was also true when compared as percent of total weight. The minimum solubility points were also very close between the fish gelatins (4.37±0.02) and the Knox® gelatin (4.34).

Two of the 300 Bloom fish gelatin and Knox® mixtures (25% and 50% fish gelatin) had higher minimum solubilities (expressed as percent of total protein content) than either the Knox® or the 300 Bloom fish gelatin whereas the sample of 75% fish gelatin had the lowest minimum solubility (see Fig. 4). All the mixtures, however, had a higher protein solubility than either the Knox® or the 300 Bloom fish gelatin at other pHs. In the case of the 200 Bloom fish gelatin which had a lower solubility (at all pH values) than Knox® gelatin, the protein solubility of the mixtures were between the two (see Fig. 5).
Fig. 3 Solubility of 5% 300 Bloom Fish Gelatin (T5104) at different pHs

Fig. 4 Solubility of the mixtures of 300 Bloom fish gelatin (T5104) and Knox® gelatin
The gel melting point seems to be concentration dependent (Fig. 6). An increase in the concentration of the gel solution, from 2% to 12%, resulted in an increase in the melting point from 21.4 to 24°C. The mean melting point of all Bloom fish gelatin samples was around 24.2±0.5°C which is 5°C lower than that of Knox® gelatin (29.6°C) (see Table 1). As with pH, melting point was significantly different, measured by one way ANOVA, from Bloom to Bloom and even within one single Bloom (300) but different lots (p<0.05). The melting point increased with the Bloom in fish gelatin samples, and although significant, the difference from the lowest Bloom (200) to the highest Bloom (300) was only 1°C (see Fig. 7).

The effect of mixing Knox® gelatin with either 200 Bloom or 300 Bloom fish gelatin is seen in Fig. 8. Since the fish gelatins have lower melting point than Knox® gelatin, it was expected that the melting point would decrease with the addition of the fish gelatin. It is interesting though that the pattern observed was not a straight line (i.e., an additive effect) but instead suggested a discontinuity, especially with 0-25% 200 Bloom fish gelatin. In addition, a second-order polynomial curve was fitted to the 300 Bloom data which coincided exactly with the experimental curve ($y = 29.614 - 3.8343x10^{-2}x - 1.4857e-4x^2$, $R^2 = 1.000$).

A linear relationship between gel strength (lb) and concentration (%) can be seen in Fig. 9 ($R^2=0.991$).
Fig. 6 Melting point as a function of concentration using 300 Bloom fish gelatin (T5104)

Fig. 7 The relationship of fish gelatin Bloom and melting point
**Fig. 8** The relationship between melting point and fish gelatin ratio in mixtures of 200 Bloom (T5113) and 300 Bloom (T5001) and Knox® gelatin

**Fig. 9** Gel Strength (lb) as a Function of Fish Gelatin Concentration (300 Bloom fish gelatin (T5104))

The results of the sensory triangle tests are shown in Table 2. In all cases, panelists were able to distinguish between the Knox® and the fish
Table 2. Sensory analysis of Knox® and fish gelatin cubes by triangle test

<table>
<thead>
<tr>
<th>Samples</th>
<th>Panelist Numbers</th>
<th>Correct Response</th>
<th>Degree of Difference</th>
<th>Preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knox® vs 300 Bloom fish gelatin (T5104)†</td>
<td>25</td>
<td>18</td>
<td>3 Slight</td>
<td>17 Knox®</td>
</tr>
<tr>
<td>Knox® vs 250 Bloom fish gelatin (T25104)†</td>
<td>17</td>
<td>13</td>
<td>3 Slight</td>
<td>8 Knox®</td>
</tr>
<tr>
<td>Knox® vs 300 Bloom fish gelatin (TA30104)†</td>
<td>17</td>
<td>14</td>
<td>3 Slight</td>
<td>7 Knox®</td>
</tr>
<tr>
<td>Knox® vs 250 Bloom fish gelatin (T25104)‡</td>
<td>24</td>
<td>19</td>
<td>7 Slight</td>
<td>11 Knox®</td>
</tr>
</tbody>
</table>

† 2.75% gelatin cubes made with cranberry juice
‡‡ 2.75% gelatin cubes made with grape juice
gelatin samples ($p<0.05$). For the 300 Bloom fish gelatin (T5104), of the panelists, who correctly identified the odd sample, all but one liked the Knox® gelatin better than the fish gelatin. Among the panelists that liked the Knox® product better, 9 panelists said that its flavor was better, 6 panelists said that both its flavor and texture were better, and 1 said that its color was better. With the 250 Bloom fish gelatin (T25104) sensory test, 8 preferred the Knox® gelatin, 5 preferred the fish gelatin, and 1 thought that neither was more acceptable, even though the fish gelatin was “softer and sweeter.” Another panelist agreed that the fish gelatin was softer, however, the panelist observed that although the flavor was the same, the texture was better in the Knox® gelatin.

In a second 300 Bloom fish gelatin (TA30104) sensory test, 7 preferred the Knox®, 6 preferred the fish gelatin and one did not have a preference but declared that the texture of fish gelatin was better, while the flavor of Knox® gelatin was better. Finally, for the 250 Bloom fish gelatin (T25104) cubes mixed with grape juice, instead of cranberry, 11 preferred the Knox® gelatin while 8 preferred the fish gelatin. Among the comments made about the fish gelatin samples were that it was sweeter and had a stronger flavor. A different aftertaste was also noted by some panelists. In summary, although Knox® gelatin was preferred among the panelists, more than 1/3 of the panelists preferred the fish gelatin cubes. The most common objection to the fish gelatin was the aftertaste which suggests that some process improvement is needed.

**CONCLUSION**

There is a clear effect of concentration, at least from 2% to 20% solutions of 300 Bloom fish gelatin, in pH, melting point and gel strength. Both melting point and gel strength increase with an increase in the concentration. The lowest pH was obtained in 10% solutions and the highest pH was obtained at the two ends of the concentration range that was used. Increasing the Bloom resulted in a small increase in the melting point, but all Bloom fish gelatin samples had a lower melting point than Knox® (about 5°C lower). The pH of Knox® (5.17) was found to be in between the pH range of the fish gelatin samples (4.98 to 5.27). There was clearly an effect of the ratio of fish gelatin in the mixtures of Knox® with 300 and 200 Bloom fish gelatin on all the properties studied (i.e., pH, melting point, and minimum solubility). The effect, however, was not additive and therefore could not be easily predicted. The minimum solubilities of the fish gelatin samples were similar to the ones obtained for Knox® expressed either as % of total protein or % of total weight. Sensory analysis showed that most panelists distinguished fish gelatin from Knox® and although there was a clear preference for the latter, more than one-third of them preferred the fish gelatin. The most
common objection to the fish gelatin cubes was the aftertaste which suggests that there is still area for improvement.

REFERENCES


Fish oil has been a significant source of fat for many people for centuries. It is widely used in Europe, the Far East and South America in such products as margarine, shortening, and cooking and salad oils. The U.S. actually consumed hydrogenated fish oil from 1921 - 1951 but this practice ceased when the California sardine fishery failed. In 1988, menhaden oil accounted for 98% of the total U.S. production of marine oils (3).

Fish oil contains the same fatty acids as vegetable oil and animal fats, plus additional long chain polyunsaturated fatty acids (C20 and C22). When hydrogenating this wide range of fatty acids, the oil crystallizes into the beta-prime form. The hydrogenation process alters the physical characteristics of the oil and gives unique functional properties. For instance, it has high crystal stability, unlike most animal and many vegetable oils. Since there is no crystal growth, it retains its smoothness and plasticity. It also has better creaming properties than vegetable oil shortening and it is resistant to "work softening" during pastry manufacturing (4).

Despite these advantages, the seafood industry has been reluctant to manufacture fish oil shortening for two reasons: 1. the shortening might have reversion ("fishy") flavors that might be imparted to the baked product (16), and 2. the shortening might be prone to develop antioxidative rancidity.
because of the high polyunsaturated fatty acid content of fish oil (18, 7, 10). However, a specially processed deodorized fish oil mayonnaise, when properly packaged and stored at 2C for 14 weeks was organoleptically acceptable and was not significantly different from soy oil mayonnaise. Moreover from chemical analyses, there were few signs of oxidation (12).

Hydrogenation decreases the tendency to oxidise and increases flavor stability (5). In an unpublished study by Stauffer et al. (unpublished study, JAOCs), refined, bleached and deodorized partially hydrogenated menhaden oil (PHMO) was used to make cookies, crackers, and deep fried extruded snacks. The PHMO items were as organoleptically acceptable as those made with partially hydrogenated vegetable oil (PHVO).

Li Hsieh and Regenstein (13) found that the best analytical tests for early oxidation of fish oil mayonnaise are peroxide value (PV) and thiobarbituric acid (TBA), while for later oxidation total carbonyl compound (TCC) and anisidine value (AV) were better.

This research is a companion work to that of Stauffer et al. (unpublished study, JAOCs) shelf-life study of cookies, crackers, and deep fried extruded snacks using sensory analysis. The objective of our work was to determine the oxidative stability of PHMO shortening in the same cookies, crackers, and deep- fried extruded snacks using PV, AV, and Total.

MATERIALS & METHODS

Raw Materials
Menhaden oil was produced by Zapata Protein Inc. It was extracted from the fish, then refined, bleached, and deodorized by standard methods. Hydrogenation, analysis, and blending of basestocks, and preparation of shortenings was carried out in the facilities of POS Pilot Plant Corporation (Saskatoon, Saskatchewan). Table 1 shows the preparation of PHMO basestocks and hardstock. Table 2 shows the production of the PHMO all-purpose shortening that was used in the cookie and cracker basecakes, PHMO filler fat that was used in the cream filling, and PHMO frying fat that was used in deep-fried extruded snacks.

The baking, deep frying, and sensory evaluation tests were done at the American Institute of Baking (AIB, Manhattan, KS). The formulas were standard ones, as used by the AIB for ingredient and process testing.

Two sets of each type of cookies, crackers, and snacks were supplied. One set was formulated with PHMO as the fat component (shortening, filler fat, spray oil, or frying fat). The other set served as the control and was formulated with a partially hydrogenated vegetable oil (PHVO) shortening.
Table 1 - Preparation of PHMO basestocks and hardstock

<table>
<thead>
<tr>
<th>Basestock #1</th>
<th>Basestock #2</th>
<th>Hardstock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>175 = 200</td>
<td>160</td>
</tr>
<tr>
<td>H₂ pressure, psi</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Ni concentration</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Time, min.</td>
<td>103</td>
<td>99</td>
</tr>
<tr>
<td>Solid Fat Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0°C</td>
<td>33.1</td>
<td>39.5</td>
</tr>
<tr>
<td>21.1°C</td>
<td>20.3</td>
<td>27.0</td>
</tr>
<tr>
<td>26.7°C</td>
<td>13.5</td>
<td>21.0</td>
</tr>
<tr>
<td>33.3°C</td>
<td>1.1</td>
<td>9.6</td>
</tr>
<tr>
<td>40.0°C</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mettler m.p., °C</td>
<td>35.8</td>
<td>38.9</td>
</tr>
<tr>
<td>Iodine value</td>
<td>76</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 2 - Production of PHMO shortenings

<table>
<thead>
<tr>
<th>All-Purpose</th>
<th>Filler Fat, Frying Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basestock #1</td>
<td>60%</td>
</tr>
<tr>
<td>Basestock #2</td>
<td>100%</td>
</tr>
<tr>
<td>Hardstock</td>
<td>10%</td>
</tr>
<tr>
<td>RBD canola oil</td>
<td>30%</td>
</tr>
<tr>
<td>Solid Fat Index</td>
<td></td>
</tr>
<tr>
<td>10.0°C</td>
<td>25.4</td>
</tr>
<tr>
<td>21.1°C</td>
<td>19.2</td>
</tr>
<tr>
<td>26.7°C</td>
<td>16.5</td>
</tr>
<tr>
<td>33.3°C</td>
<td>11.1</td>
</tr>
<tr>
<td>40.0°C</td>
<td>3.6</td>
</tr>
<tr>
<td>Mettler m.p., °C</td>
<td>43.6</td>
</tr>
<tr>
<td>Smoke point, °C</td>
<td>248</td>
</tr>
<tr>
<td>AQM, hrs.</td>
<td>162</td>
</tr>
</tbody>
</table>
Two types of cookies were tested: a rotary sandwich cookie with a cream filling, and a wire cut butter crunch cookie. The experimental sandwich cookie and butter crunch cookie basecake were made with all-purpose PHMO shortening (see Table 2); the control cookie basecake used partially hydrogenated soybean/cottonseed oil shortening (BBS-C, Capital City Cc., Karlshamns, Columbus, OH). The experimental sandwich cookie cream filling was made with PHMO filler fat and the control was made with partially hydrogenated coconut oil filler fat (PUR-CO 92, Capital City Cc.).

Snack crackers used all-purpose PHMO shortening in the experimental cracker and were sprayed with all-purpose PHMO. Control crackers contained partially hydrogenated soybean/cottonseed oil shortening and sprayed with hydrogenated canola oil (Lobra 70, Capital City Co.). The spray oil, 12-15%, was applied to the hot crackers as they exited the oven.

Experimental extruded snack pellets were deep fried in PHMO frying fat shortening while the control snacks were deep fried in all vegetable frying fat (XXX Vcream, Bunge Corp.). Extruded snack pellets were fried in a floor model fryer (Filter Magic, Fry Master Corp., Shreveport, IA) at 204C for 10 sec.

Storage

All test material were stored at room temperature. Since the cookies and crackers were packed (by the supplier) in clear plastic bags, they were exposed to fluorescent light and some sun light.

Sensory evaluation

Sensory evaluation of the product was conducted using 20 untrained panelists. Products were tested using a triangle test at 0, 1, 2, 4, and 6 months after baking. Panelists were asked to taste the samples in the order indicated and identify the odd sample. They were also asked to indicate the degree of difference between the samples and the acceptability of each.

Peroxide value (PV)

This is the most widely used method to determine the degree of oxidation (17). The primary oxidation products of oils and fats are hydroperoxides, the quantity of which can be quantitatively measured by determining the amount of iodine liberated by its reaction with potassium iodide. The peroxide content is expressed in terms of milliequivalents of iodine per kilogram of oil or fat.

Fat extraction for the determination of PV was done by the procedure of Ke and Woyewoda (11). The PV for the extracted fat was determined by A.O.C.S. official methods (1, 2).
Anisidine value (AV)

Anisidine value measures the later oxidation of fat by measuring alpha-beta unsaturated aldehydes of fats and oils. This method of determining the degree of oxidation is extensively used in European countries (17).

To measure anisidine values, the method for fish oil as published in the International Association of Fish Meal Manufacturers (IARMM) Fish Oil Bulletin (8) was used.

Totox

An extension of these methods is the Totox value, which is a measure of total oxidative deterioration, with an emphasis on primary oxidation. Totox is the sum AV and twice the PV (6, 9, 17).

Statistical analysis

The general linear model of Minitab 7.2 (Minitab, Inc., State College, PA) was used to do the analysis of variance, analysis of covariance, and regression, since it can be used with unbalanced designs and missing data.

For each test, analysis of variance with respect to time, treatment and the interaction of time and treatment was determined (p<0.05). In order to determine significance (p<0.05) between overall means of treatments, a one-way analysis of variance was done with time averaged out.

RESULTS & DISCUSSION

Sensory evaluation

Sandwich cookies. At 0 and 1 month, panelists could detect a slight difference, although not at the 5% significance level. By the 6th month, 0 panelists picked the correct odd sample. With regard to preference, the panelists seemed to slightly prefer the control sample, but the split was so small as to be considered random. Again, results of the triangle test indicated that there was no significant difference between sandwich cookies made with PHMO as both the basecake and the filling and the control cookie.

Butter crunch cookies. For the triangle test, with 20 panelists, 11 correct responses corresponds to a 5% significance level. This was reached only when the cookies were evaluated after 2 months of storage Table (Table 3). On the other test times, the selections appeared to be random. Of the panelists who correctly identified the sample, the panelists' preference, also appear to be random, except for perhaps the 2nd month, where 7 out of 12 preferred the PHMO cookies. Results of the taste panel tests indicated that there was no significant difference between the experimental butter crunch cookie and the control cookie.
Table 3 - Sensory analysis\(^a\) of sandwich cookies over time by triangle test

<table>
<thead>
<tr>
<th>Months</th>
<th>Correct Response</th>
<th>preference (Of Correct Responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish Oil Control</td>
<td>Neither</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)20 untrained panelists: 5% significance requires 11 correct responses.

Table 4 - Sensory analysis\(^a\) of butter crunch cookies over time by triangle test

<table>
<thead>
<tr>
<th>Months</th>
<th>Correct Response</th>
<th>preference (Of Correct Responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish Oil Control</td>
<td>Neither</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)20 untrained panelists: 5% significance requires 11 correct responses.

Table 5 - Sensory analysis\(^a\) of snack crackers over time by triangle test

<table>
<thead>
<tr>
<th>Months</th>
<th>Correct Response</th>
<th>preference (Of Correct Responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish Oil Control</td>
<td>Neither</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>-</td>
</tr>
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<td>4</td>
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<td>2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)20 untrained panelists: 5% significance requires 11 correct responses.

Table 6 - Sensory analysis\(^a\) of deep-fried extruded snacks over time by triangle test

<table>
<thead>
<tr>
<th>Months</th>
<th>Correct Response</th>
<th>preference (Of Correct Responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish Oil Control</td>
<td>Neither</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\)20 untrained panelists: 5% significance requires 11 correct responses.
Snack crackers. Only at 0 and 1 month, could the panelists detect a significant difference between the experimental and test crackers with a preference toward the control crackers (Table 5). Comments indicated that some of the panelists could pick up a slight fishy taste. As the crackers "aged", the differences between the crackers seemed to decrease.

Deep-fried extruded snacks. If an oil is prone to autoxidative rancidity and development of reversion flavors, using it to make a deep-fried extruded snack item is a stringent test. The snacks were not coated with any flavoring materials, so the panelists were experiencing the crunchy texture of the bland substrate with the frying oils as the only source of flavor. As shown in Table 6, the ability of the panelists to detect the correct odd sample did not reach significance during the entire period of the study. At the beginning of the study, there was a slight preference for the control samples. However, this does not seem to be significant in view of the undetectable difference between the PHMO and control samples.

**Biochemical evaluation**

Since Totox includes contributions from both AV and PV, only the Totox data will be presented.

**Sandwich cookies.** The Totox of the sandwich cookie basecake and the cream filling made with PHMO shortening was slightly higher than the control throughout most of the study (Fig. 1). However, except for the initial period, both fluctuated at fairly low levels (as compared to high-quality crude soybean oils, which will generally have a Totox value of less than 3; for crude soybean sunflower oil, the value is generally less than 5 (15). The differences in Totox between the experimental and control sandwich cookies were nonsignificant for both the cookie and cream layers.

**Wire cut butter crunch cookies.** Totox values for the control cookies were higher than the experimental cookies at 2 and 8 months; and lower at 4 and 6 months (Fig. 2). The differences in Totox for butter crunch cookies were nonsignificant. Totox values were acceptable, since for the most part, the values were under 5.

**Snack crackers.** Totox in PHMO snack crackers were generally higher than the controls with a fair bit of scatter, but the differences generally decreased with time (Fig. 3). By the end of the study the differences were slight. Also, except for 0 and 6.5 months, the Totox values for PHMO snack crackers showed good stability, since the values were less than 5. The differences in Totox for snack crackers were significant. However, by the end of the study (8.5 months) the Totox values were essentially the same. Also, with the PV test, there was no significant difference.
Fig. 1 - The effect of time on rancidity of sandwich cookies

Fig. 2 - The effect of time on rancidity of butter crunch cookies
Fig. 3 - The effect of time on rancidity of snack crackers

Fig. 4 - The effect of time on rancidity of extruded snacks
Deep-fried extruded snacks. PHMO deep-fried extruded snacks had higher initial Totox, but by the 6.5 month, the Totox was lower than the control (Fig. 4). The data for the 2nd month was missing, therefore a dashed line was used to connect the 0 month and the 4th month. Both fluctuated at fairly low levels. With Totox, there was no significant difference between PHMO and the control extruded snacks.

CONCLUSION

Data from the taste panels indicate that there are no significant differences between PHMO shortening cookies, snacks and deep fried extruded snacks; and those made with control PHVO shortening (except for the butter crunch cookies at the 2nd month, and snack crackers at 0 and the 1st month.) For the most part, differences between the products decreased with increasing shelf-life.

No significant difference for Totox was found when PHMO shortening cookies, crackers, and deep-fried extruded snacks were compared to similar items made with PHVO shortening (except the AV and Totox of snack crackers, and Totox of sandwich cookies). Since there was little difference between the oxidative stability of the PHMO cookies, crackers, and deep-fried extruded snacks and the controls, PHMO shortening may be used in cookie, cracker, and deep-fried extruded snack formulations without concerns about rancidity.

These results showed that there was little difference between the oxidative stability of the fish oil cookies and crackers and controls, therefore hydrogenated menhaden fish oil may be used in cookie, cracker, and deep-fried extruded snack formulations without concerns about rancidity.
REFERENCES


Sodium or potassium lactate, a salt of lactic acid, is a Generally Regarded As Safe (GRAS) chemical. It is often used in bakery products including biscuits, cakes and confectionery to keep them moist and give them a soft, crumbly texture, and in cheese to enhance the action of antioxidants. It is also used in jam and marmalade, and margarine to regulate the acidity. In recent years, sodium lactate has been used in meat products as a flavor enhancer and an antimicrobial agent. However, due to the fact that consumers have a trend to reduce the sodium intake, potassium lactate has been used to partially substitute sodium salt.

Previous studies indicated that sodium lactate inhibited microbial growth and prolong the shelf life of meat products. Duxbury (4) reported that beef roast injected with sodium lactate resulted in increased shelf life of the roasts. Bacterial growth was inhibited and the microflora came to be dominated by Lactobacillus species. Chirife and Fontan (2) stated that sodium lactate has been shown to decrease the water activity sufficiently to inhibit bacterial growth. de Witt and Rombouts (3) reported that the antimicrobial effects of sodium lactate may due to the increased permeability of cellular membranes for lactate ion at a high pH. However, feedback inhibition by lactate was proposed as the mechanism of action in the delay of toxin production by Streptococcus faecalis (8) and by Clostridium botulinum (5).

Little information regarding the effect of sodium lactate or potassium lactate on fishery products was available. Williams and Rodrick (10) reported that sodium lactate functioned to retard microbial growth, and stabilize color and odor characteristics in the skinned catfish fillets. Total aerobic plate counts of cod fillets were also big reduced when 12% of sodium lactate solution was used to immerse the fillets for 2 min (1). However, no study of sodium lactate or a mixture of sodium and potassium lactates on tilapia has been made. In order to extending the shelf life of fresh product at the retail level, the objectives of this study were to determine the reduction of microbial populations on tilapia fillet treated with sodium lactate or a mixture of sodium and potassium lactates.
MATERIALS AND METHODS

Tilapia

Tilapia (Orechromis spp.), purchased from the Delkab Farmers Market (Decatur, GA). The fish were headed, gutted and filleted and then transported on ice to the University of Georgia, Athens, GA. The tilapia fillets were treated with 8% sodium lactate or a mixture of 4% sodium lactate and 4% potassium lactate solutions for 2 min.

Treatments

All treated fillets were packaged by either overwrapping with polyvinylidene films (Saran wrap, oxygen transmission rate: 5 cc/m²/24 hr/atm at 20°C and 43% RH), or vacuum-skin packaging with low density polyethylene films (Surlyn, oxygen transmission rate: 930 cc/m²/24 hr/atm at 23°C and 75% RH) using a Trigon Intact RM 33 Mini Mark III Mini Intact machine (Trigon Packaging Corp., Redmond, WA), or modified atmosphere packaging with 70% CO₂ and 30% N₂. Packaged fish were stored at a 4°C walk-in cooler for 16 days. Three packages of each treatment were randomly removed from cooler at a 4-day interval for determination of pH, color values (L, “a”, “b”), Torrymeter readings and microbial counts.

Microbiological Evaluation

A 5x5 cm section of fillet was collected by using a sterilized blade. The sections were then placed in stomacher bags with 100 ml of 0.1% peptone buffer solution (Bacto) and stomached for 2 min by a Stomacher (Seward model 400, London, England). The aliquots of proper dilution were plated onto plate count agar and incubated at 20°C for 4 days to enumerate a total psychrotrophic bacterial count (9). The aliquots were also plated onto trypticase peptone glucose yeast extract agar (TPGY) and incubated at 20°C for 4 days to enumerate total anaerobic plate counts (9). The BBL GasPak® anaerobic chamber with BBL GasPak® CO₂ gas packs (Becton Dickinsin Microbiology Systems, Boston, MA) were used to create an anaerobic environment for incubation.

Surface pH Measurement

Triplicate pH readings were taken from the surface of each sample fish by using a Fisher Scientific probe (No. 13-620-286) and a Corning pH meter (model Y140, Corning Inc., NY, NY).

Color Analysis

The color of fish skin surface was measured prior to removal of the core sample by using a Colormeter (Minolta CR-200, Osaka, Japan). Hunter color values of L (lightness), “a” (“+” : redness; “-” : greenness) and “b” (“+” : yellowness; “-” : blueness) were recorded. Triplicate measurements were taken from each fish.
Torrymeter readings

Six spots of fillet were measured using a Torrymeter (gr Torry Station, Scotland, UK). The reading ranges from 1 to 16.

Data Analysis

Statistical analyses (6) were performed on pH, color values, Torrymeter readings and microbiological data by means of PC SAS. The Duncan's multiple range test was used to determine any significant differences among samples from fish with different treatments.

RESULTS AND DISCUSSION

Effects of lactates treatment and packaging system on psychrotrophic bacterial growth on tilapia

On day 8, control samples and overwrapped samples treated with either 8% sodium lactate or the mixture of 4% sodium lactate and 4% potassium lactate exhibited obvious spoilage, while modified atmospheres packaged ones had significantly lower bacterial counts (Table 1). Vacuum skin packaged fillets treated either sodium lactate or the mixture exhibited an onset of spoilage. No significant differences between vacuum skin packaged samples or between MAP samples were found regardless of chemical treatment. Results indicated that 8% sodium lactate had the same effect as the mixture of sodium lactate and potassium lactate. Modified atmosphere packaging significantly retarded microbial growth on tilapia and prolong the shelf life for two times.

The same growth pattern of anaerobic bacteria as psychrotrophic bacteria was found in tilapia (Table 2). Modified atmosphere packaging did not promote the growth of anaerobic bacteria on lactates treated tilapia.

Effects of lactates treatment on surface pH of tilapia as affected by packaging system

Both lactates treatment significantly reduced the initial pH on tilapia skin surface and this effect last till day 8 (Table 3). On day 12, no significant difference on pH among samples was found except the MAP samples. This was because that all the samples were spoiled except the MAP ones. The absorption of carbon dioxide by the fish was another factor to cause the lower pH value.

Effects of lactates treatment and packaging system on color of tilapia fillet

No clear difference on Hunter color values of all samples was found regardless of sodium lactate treatment and packaging system (Tables 4, 5, and 6). Results implied that neither lactates treatment nor packaging system had no effect on Hunter color values of tilapia fillets.
### Table 1. Psychrotrophic bacteria count (logCFU/cm²) of tilapia as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>VSP</td>
<td>M A P</td>
</tr>
<tr>
<td>control</td>
<td>2.69a</td>
<td>2.69a</td>
<td>2.69a</td>
</tr>
<tr>
<td>4</td>
<td>5.55a</td>
<td>4.98ab</td>
<td>4.60bc</td>
</tr>
<tr>
<td>8</td>
<td>8.82a</td>
<td>8.13b</td>
<td>7.20c</td>
</tr>
<tr>
<td>12</td>
<td>9.15a</td>
<td>9.09a</td>
<td>8.53b</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
<td>6.24a</td>
</tr>
</tbody>
</table>

OW VSP MA P OW VSP MA P

2.69a 2.69a 2.69a 2.69a 2.69a 2.69a 2.69a 2.69a
2.69a 2.69a 2.69a 2.69a 2.69a 2.69a 2.69a 2.69a

Means in a column followed by the same letter are not significantly different at level of 0.05.

### Table 2. Total anaerobic bacteria count (logCFU/cm²) of tilapia as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>VSP</td>
<td>M A P</td>
</tr>
<tr>
<td>control</td>
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<td>2.64a</td>
<td>2.64a</td>
</tr>
<tr>
<td>4</td>
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<td>4.02c</td>
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<td>8</td>
<td>8.82a</td>
<td>8.13b</td>
<td>7.64c</td>
</tr>
<tr>
<td>12</td>
<td>10.26a</td>
<td>9.85ab</td>
<td>9.68ab</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
<td>6.06a</td>
</tr>
</tbody>
</table>

OW VSP MA P OW VSP MA P

2.64a 2.64a 2.64a 2.64a 2.64a 2.64a 2.64a 2.64a
4.25bc 3.80c 2.59d 4.25bc 3.80c 2.59d 4.25bc 3.80c

Means in a column followed by the same letter are not significantly different at level of 0.05.

### Table 3. Surface pH of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>VSP</td>
<td>M A P</td>
</tr>
<tr>
<td>control</td>
<td>6.78a</td>
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<td>6.43b</td>
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<td>6.47ab</td>
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<tr>
<td>8</td>
<td>6.75a</td>
<td>6.44b</td>
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<td>12</td>
<td>6.95ab</td>
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<td>6.78abcd</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
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</table>

OW VSP MA P OW VSP MA P

6.46b 6.46b 6.46b 6.46b 6.46b 6.46b 6.46b 6.46b
6.39b 6.49ab 6.34b 6.39b 6.49ab 6.34b 6.39b 6.49ab

Means in a column followed by the same letter are not significantly different at level of 0.05.
Table 4. Hunter L values of tilapia on shin side as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>VSP</td>
<td>MAP</td>
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<tr>
<td>0</td>
<td>52.72a</td>
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<td>53.09a</td>
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<td>4</td>
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<td>52.98ab</td>
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<tr>
<td>8</td>
<td>52.98a</td>
<td>55.35a</td>
<td>53.77a</td>
</tr>
<tr>
<td>12</td>
<td>55.34abc</td>
<td>58.36a</td>
<td>53.31bc</td>
</tr>
<tr>
<td>16</td>
<td>57.74a</td>
<td>57.73a</td>
<td>49.95b</td>
</tr>
</tbody>
</table>

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

Table 5. Hunter "a" values of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>VSP</td>
<td>MAP</td>
</tr>
<tr>
<td>0</td>
<td>-0.86a</td>
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<td>-1.29a</td>
</tr>
<tr>
<td>4</td>
<td>-0.89a</td>
<td>-1.38abc</td>
<td>-1.23abc</td>
</tr>
<tr>
<td>8</td>
<td>-1.75b</td>
<td>-1.86b</td>
<td>-1.58ab</td>
</tr>
<tr>
<td>12</td>
<td>-2.26b</td>
<td>-1.56bc</td>
<td>-1.41ab</td>
</tr>
<tr>
<td>16</td>
<td>-1.67abc</td>
<td>-0.97a</td>
<td>-1.09a</td>
</tr>
</tbody>
</table>

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

Table 6. Hunter "b" values of tilapia on shin side as affected by lactates treatment and packaging system at 4°C

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>control</th>
<th>8% sodium lactate</th>
<th>mixture</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>OW</td>
<td>VSP</td>
<td>MAP</td>
</tr>
<tr>
<td>0</td>
<td>-4.95a</td>
<td>-3.97a</td>
<td>-3.97a</td>
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<td>4</td>
<td>-2.20ab</td>
<td>-3.57b</td>
<td>-3.67b</td>
</tr>
<tr>
<td>8</td>
<td>-0.87a</td>
<td>-2.03ab</td>
<td>-3.34bc</td>
</tr>
<tr>
<td>12</td>
<td>-0.90a</td>
<td>-0.70a</td>
<td>-2.58a</td>
</tr>
<tr>
<td>16</td>
<td>-1.29a</td>
<td>-1.69a</td>
<td>-2.72a</td>
</tr>
</tbody>
</table>

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05
In summary, at 4°C the over-wrapped tilapia fillet had shelf life of 6 days with any lactate treatment. Significant effects on microbial growth were found for fish treated with the combination of modified atmosphere packaging and dipping in the 8% sodium lactate solution or in the mixture of 4% sodium lactate and 4% potassium lactate. Vacuum skin packaging had also shown a reduction for psychotropic bacteria growth, while the over-wrapping had only little effect.

ACKNOWLEDGMENTS

The study was supported, in part, by the Georgia Agriculture Experiment Station, the University of Georgia and the Southern Regional Aquaculture center, USDA

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EFFECTS OF TANNIC ACID, GALLIC ACID, AND PROPYL GALLATE ON STORAGE LIFE OF CATFISH

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Athens, GA 30602-7610, USA
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Memphis State University
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and
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I-Lan, Taiwan ROC

Tannins are present in many plant products including strawberries, grapes, apples and tea. The substances are categorized as phenolic compounds and can be divided into two categories: hydrolyzable and condensed tannins. The hydrolyzable tannins can be further divided into two classes: gallotannins and ellagitannins. Upon hydrolysis, the gallotannins yield gallic acid and glucose while ellagitannin yield ellagic acid and sucrose.

Tannic acid has been used as a clarifier for many years. It is also used to refine fats. Tannic acid is of amorphous powder, glistening scales, spongy masses. It exhibits yellow, white, and brown colors. It is usually odorless but occasionally with a faint odor, and with an astringent taste.

On the antimicrobial activity, tannic acid is inhibitory to many foodborne bacteria including Aeromonas hydrophila, Escherichia coli, Listeria monocytogenes, Salmonella enteritidis, Staphylococcus aureus, and Streptococcus faecalis while gallic acid and ellagic acid are not. When tannic acid is hydrolyzed, the ester bond is broken to release gallic acid and/or ellagic acid and the antimicrobial activity is lost (1).

The antimicrobial activities of tannin include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (4).

Propyl gallate is the Common name for n-propyl 3, 4, 5 trihydroxybenzoate. Blending BHA with propyl gallate shows particularly efficacious in preserving flavor of oils and fats. Propyl gallate discolors when in the presence of iron or copper, unless it is treated with citric acid to form citric acid metal ion compounds to reduce discoloration.
Propyl gallate is a GRAS antioxidant, white or off white powder. It is odorless and has slightly bitter taste. Legal requirements for antioxidants and oxygen interceptors permitted in meat and poultry processing: to retard rancidity rendered animal fat or a combination of such fat and regular fat: 0.01%; 0.02% in combination. For dried Meats, 0.01% based on total weight are suggested (2).

The objectives of this study were to determine the effects of tannic acid, gallic acid, and propyl gallate on psychrotrophic plane count of over-wrapped catfish fillets, and to evaluate the color and surface pH changes during refrigerated storage for 12 days.

MATERIALS AND METHODS

Catfish

Fresh farmed catfish (Ictalurus punctatus) were purchased from the Dekalb Farmers Market, Decatur, Georgia. Fish were headed, eviscerated, skinned, and filleted at the market’s facilities. The fillets were transported on ice to the Food Science Department, University of Georgia in Athens and then the ice chests were placed in the 4°C walk-in cooler.

Treatment

Tannic acid, gallic acid and propyl gallate were purchased from the Sigma Co. (St. Louis, MO). One percent tannic acid (TA), 1% gallic acid, and 1% propyl gallate were used to treat fillets. One hundred pieces of fillets were randomly assigned to the treatments. These treatments had used ice to cool the temperature down close to 0°C to minimize fish spoilage. Fillets were dipped in the appropriate treatment solutions for 30 minutes and drained before packaging procedures.

Packaging and Storage

The treated fillets were placed on polystyrene trays (1 fillet per tray) and overwrapped with Saran® (PVDC, polyvinylidene chloride, oxygen transmission rate = 5 cc/m²/24hr/atm, at 20°C, 43% RH.; Dow Chemical Co., Indianapolis, IN). All packaged fillets were stored in a 4°C walk-in cooler until sampling days on day 0, 4, 8, and 12. Three packages of each sample were used for all the tests.

Microbiological Analysis

A size of 25 cm², from the central portion of fillets, was obtained and individually homogenized for 30 seconds with 100 ml sterile 0.1% peptone solution in Fisher polyethylene sample bags using Seward (England) Laboratory Blender Stomacher 400. Serial dilutions were further made in 0.1% peptone, and suitable dilutions were plated by spreading 0.1 ml onto the surface of Plate Count Agar (Difco, Detroit, MI). Psychrotrophic plate counts were made in duplicate. Plates were incubated for 48 hours at 20°C. The results were expressed as the log₁₀ of cfu/cm².

Color Measurements

The Hunter L, “a”, “b” were measured by using a Chroma Meter CR200 (Minolta Camera Ltd., Osaka, Japan). The value L measures lightness which range from 0 to 100; “a” measures chromaticity where positive value indicating redness and negative value indicating greenness; while
positive value of “b” indicating yellowness and negative value indicating blueness. Color measurements were made with the packaging unremoved to avoid the effect of packaging material. Three random spots were measured and the average data were recorded.

**pH Measurement**

Surface pH was measured using a Corning Ml40 pH meter (Corning Medical & Scientific Instruments, Halstead Essex, England). pH was measured on three random spots on each fillet and the average reading was recorded.

**Statistical Analysis**

Analysis of variance was performed on the data by means of PC SAS. The Duncan’s multiple range test was used to determine if any significant difference among samples as affected by packaging method and different additive treatment.

**RESULTS AND DISCUSSION**

The only significant difference in psychrotrophic plate count on Day 4 was displayed by the sample treated with propyl gallate (Table 1). However, no significant difference among samples were found at the end of 12 days storage. Looking at the pH changes, samples treated with gallic acid and tannic acid were found to have significant lower pH than control and propyl gallate treated samples (Table 2). There were no significant difference between propyl gallate treated samples and control.

Table 1. **Psychrotrophic** plate counts (log CFU/cm²) of over-wrapped catfish fillets as affected by different additives during refrigerated storage

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>Control</th>
<th>Tannic acid</th>
<th>Gallic acid</th>
<th>Propyl gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.45(0.10)a</td>
<td>4.23(0.24)a</td>
<td>4.10(0.71)a</td>
<td>3.74(0.24)a</td>
</tr>
<tr>
<td>4</td>
<td>6.94(0.14)a</td>
<td>6.95(0.57)a</td>
<td>6.32(0.57)a</td>
<td>5.49(0.12)b</td>
</tr>
<tr>
<td>8</td>
<td>8.41(0.13)a</td>
<td>8.51(0.30)a</td>
<td>8.14(0.09)a</td>
<td>7.54(0.33)b</td>
</tr>
<tr>
<td>12</td>
<td>8.79(0.21)a</td>
<td>8.65(0.37)a</td>
<td>8.70(0.11)a</td>
<td>8.56(0.15)1a</td>
</tr>
</tbody>
</table>

Means in a row with the same letter are not significantly different at p=0.05 (n=3)
Table 2. Surface pH of over-wrapped catfish as affected by different additives during refrigerated storage

<table>
<thead>
<tr>
<th>Day of Control storage</th>
<th>Tannic acid</th>
<th>Gallic acid</th>
<th>Propyl gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.56(0.16)a</td>
<td>5.66(0.25)b</td>
<td>5.05(0.20)c</td>
</tr>
<tr>
<td>4</td>
<td>6.83(0.08)a</td>
<td>6.39(0.14)b</td>
<td>6.02(0.12)c</td>
</tr>
<tr>
<td>8</td>
<td>6.82(0.08)a</td>
<td>6.89(0.77)a</td>
<td>6.41(0.11)a</td>
</tr>
<tr>
<td>12</td>
<td>6.97(0.14)b</td>
<td>6.79(0.04)b</td>
<td>6.70(0.26)b</td>
</tr>
</tbody>
</table>

Means in a row with the same letter are not significantly different at p=0.05 (n=3)

The color of the catfish fillets turned lighter (as observed with higher L values) with chemical additives treatment (Table 3). The fillets treated with tannic acid exhibited significant lower “a” (Table 4) and higher “b” (Table 5) values in over-wrapped samples, indicating that the treated samples were of green and yellow tinge as compared to the control samples. On the other hand, samples treated with propyl gallate showed no significant difference in “a” and “b” values as compared to the controls.

Table 3. Hunter L value of overwrapped catfish as affected by different additives during refrigerated storage

<table>
<thead>
<tr>
<th>Day of Control storage</th>
<th>Tannic acid</th>
<th>Gallic acid</th>
<th>Propyl gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55.12(3.84)c</td>
<td>56.38(2.12)c</td>
<td>69.46(0.89)a</td>
</tr>
<tr>
<td>4</td>
<td>56.38(2.12)c</td>
<td>65.43(1.46)a</td>
<td>61.68(2.27)b</td>
</tr>
<tr>
<td>8</td>
<td>55.42(2.56)b</td>
<td>60.38(1.51)a</td>
<td>61.27(1.47)a</td>
</tr>
<tr>
<td>12</td>
<td>58.45(1.89)a</td>
<td>59.85(2.49)a</td>
<td>57.80(6.73)a</td>
</tr>
</tbody>
</table>

Means in a row with the same letter are not significantly different at p=0.05 (n=3)
Table 4. **Hunter “a” value of overwrapped** catfish as affected by different additives during refrigerated storage

<table>
<thead>
<tr>
<th>Day of Control storage</th>
<th>Tannic acid</th>
<th>Gallic acid</th>
<th>Propyl gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-1.43(0.48)a</td>
<td>-2.60(0.37)b</td>
<td>-1.88(0.24)a</td>
</tr>
<tr>
<td>4</td>
<td>-1.51(0.24)a</td>
<td>-2.20(0.03)b</td>
<td>-1.92(0.20)a</td>
</tr>
<tr>
<td>8</td>
<td>-1.49(0.49)a</td>
<td>-1.99(0.57)a</td>
<td>-2.16(0.18)a</td>
</tr>
<tr>
<td>12</td>
<td>-2.51(0.17)a</td>
<td>-2.14(0.32)a</td>
<td>-2.70(0.34)a</td>
</tr>
</tbody>
</table>

Means in a row with the same letter are not significantly different at p=0.05  (n=3)

Table 5. Hunter “b” value of overwrapped catfish as affected by different additives during refrigerated storage

<table>
<thead>
<tr>
<th>Day of Control storage</th>
<th>Tannic acid</th>
<th>Gallic acid</th>
<th>Propyl gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-2.56(0.62)b</td>
<td>0.15(4.55)a</td>
<td>-3.24(0.40)b</td>
</tr>
<tr>
<td>4</td>
<td>-2.09(0.61)b</td>
<td>4.35(0.35)a</td>
<td>-1.74(0.41)b</td>
</tr>
<tr>
<td>8</td>
<td>-0.48(1.40)b</td>
<td>3.85(1.40)a</td>
<td>-1.44(0.46)b</td>
</tr>
<tr>
<td>12</td>
<td>-0.13(2.41)bc</td>
<td>4.17(0.74)a</td>
<td>1.60(0.57)bc</td>
</tr>
</tbody>
</table>

Means in a row with the same letter are not significantly different at p=0.05  (n=3)

The psychrotrophic plate count of the water of the fish tank (in Dekalb’s Farmer Market) was found to be log 5 cfu/g which is higher than normal. Leung et al (3) reported an initial psychrotrophic plate count of log 2.45 cfu/cm² on channel catfish harvested from the Fisheries Research Unit, Auburn University, Auburn Alabama. Therefore, the initial psychrotrophic plate counts of catfish fillets for this study was higher, contributing to a shorter shelf-life, that is about 4 days. Color changes of fillets as affected by chemical is undesirable for consumers’ acceptance of the product. Therefore, propyl gallate could be used without affecting the color changes of the fillets.
In summary, fillets treated with propyl gallate had significantly lower psychrotrophic plate counts on day 4 and 8 as compared to the control. pH of fillets treated with tannic acid and gallic acid was significantly lower than that of control and fillet treated with propyl gallate. All treated samples had significantly lighter than control, while samples treated with tannic acid had significantly higher “b” value (yellowness) and higher “-a” value (greenness) than other samples. In general, the propyl gallate showed the potential to reduce the psychrotrophic bacteria counts on catfish fillet. However, further studies on sensory evaluations have to be done in the future.

ACKNOWLEDGMENTS

This study was supported, in part, by the Georgia Agriculture Experiment Station, the University of Georgia, and the Southern Regional Aquaculture Center, USDA.

REFERENCES


