This is to certify that the

thesis entitled

Storage Stability of Mechanically Deboned Fish Flesh

presented by

David Michael Morris

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Food Science & Human Nutrition

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Date 9-30-77

O-7639

STORAGE STABILITY OF MECHANICALLY DEBONED FISH FLESH

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1977

ABSTRACT

STORAGE STABILITY OF MECHANICALLY DEBONED FISH FLESH

By

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The 2-thiobarbituric acid (TBA) test was used to evaluate the development of rancidity in mechanically deboned freshwater mullet treated with various chemical and physical treatments. After six months of frozen storage, samples containing Tenox A^{R}, Tenox II^{R}, and Tenox PG^{R} had significantly higher TBA values than did those treated with Freez-Gard^{R}.

Analyses of patties made from deboned mullet showed that TBA values of Freez-Gard treated samples were about 50% lower than controls. Sensory evaluations indicated that the addition of Freez-Gard improved flavor and increased firmness of the patties. The presence in the flesh of a binder preparation, containing phosphate, ascorbate, nitrite, and other ingredients resulted in lower TBA values. A monoglyceride film helped to slow down TBA development in patties but sensory scores indicated that the appearance, flavor, and texture were undesirable. Vacuum packaging and, to a lesser degree, precooking resulted in a lower rate of oxidative change.

ACKNOWLEDGMENTS

I would like to extend my appreciation to Dr. L.E. Dawson for his patience and guidance throughout this study program. I am also grateful to the members of my committee for their academic support and to the department for financial support.

I would like to extend thanks to Mark and Kit Uebersax for their willingness to answer my multitude of questions and their patience and direction in the laboratory.

Special thanks are given to Mary Vallender for her constant support, encouragement, and friendship.

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INTRODUCTION

Productivity of the commercial fishing industry in the Great Lakes region has substantially decreased in recent years. This is due to the reduction of stocks of lake trout and other species by the invasion of the sea lamprey and overfishing causing a decline in the stocks of chubs and whitefish. Also contributing to the decline of the commercial fish harvest is the regulatory ban on large mesh gill nets and regulatory emphasis designed for the development of the sport fishing industry, often at the expense of the commercial fish industry. Freshwater mullet, (Catostomus commersoni), is an abundant under-utilized species and is a usable food source; but an excessive number of small bones prevent it from being used in a fillet form. Using mechanical deboning equipment, a minced fish product, excluding these small bones, can be produced.

The functional characteristic of the mechanically deboned fish will allow the minced flesh to be incorporated into several traditional food products as well as allow for the development of new products. Possible uses include formulation into fish portions, sausage, croquettes, casseroles, chowder, or a variety of other products. Mechanical deboning is advantageous because resources are used more

efficiently, yields are increased, and there is greater flexibility in processing.

Mechanically deboned fish flesh is a good source of several nutritional components. It is a food high in protein and a relatively high content of polyunsaturated fatty acids, which may be helpful in lowering or maintaining serum cholesterol at desirable levels. The favorable mineral content of fish helps to up-grade its nutritional quality.

Increased oxidation rate and rapid development of rancid flavors prevents minced fish flesh from being as acceptable as whole fish fillets. Fish products oxidize more rapidly and reactions are more complicated than in other foods, mainly because of the high proportions of unsaturated fatty acids. Mechanical deboning increases surface area and incorporates oxygen into the product. This hastens the development of oxidative rancidity and results in formation of the low molecular weight by-products of oxidation and the characteristic off-flavor of rancid products. To delay the development of oxidative rancidity, various chemical and physical treatments were used to produce a more desirable product. This experiment was conducted to provide information relating to the control of rancidity in raw and precooked fish patties with and without vacuum packaging. Antioxidants, a binder, and an edible film were also used to establish which treatment allowed the longest frozen storage life of breaded patties.

Specific objectives were:

(1) To determine the effect of various antioxidants on the oxidation of lipids in minced fish flesh during frozen storage;

(2) To evaluate the effect of an antioxidant, a binder system, and a monoglyceride film on the lipid stability and flavor changes in breaded fish patties during frozen storage;

(3) To ascertain the effect of vacuum packaging on raw and precooked fish patties on the development of oxidative rancidity;

(4) To evaluate the interaction effect of antioxidant treatments, precooking, and vacuum packaging as related to oxidation and flavor of frozen breaded fish patties.

LITERATURE REVIEW

Mechanical Deboning

Equipment and Process

About twenty years ago mechanical deboning machinery was developed and is being used today by the poultry industry to increase yields of edible meat. More recently the fish industries in the United States have begun to evaluate mechanical deboning as a means to increase the amount of available edible fish flesh.

Fish that are to be mechanically deboned must first be headed, gutted, and split. The racks remaining after conventional filleting may also be used. The fish are then fed into the machine between a belt and a perforated drum with 2 to 7 mm holes. The belt and drum move in the same direction but at different speeds creating shear stresses. The flesh is torn from the skin and bones and the pressure applied by the belt forces the fish through the perforations in the drum. The skin and bones are scraped off the drum and discarded through a waste chute. The pressure of the belt can be adjusted to remove only the light meat during a first pass and by increasing the belt pressure, the dark meat closer to the skin can be removed during a second pass. Maximum belt pressure will remove both light and dark meat

with one pass.

Yields of Mechanical Deboning Equipment

Reported yields of mechanically deboned headed, gutted, and split fish, based on the weight of the whole fish, 37-60% (Miyauchi and Steinberg, 1970), 40-86% (Carver and King, 1971), 48-60.2% (Crawford et al., 1972a), 40-54.5% (Crawford et al., 1972b), 35-95% (Miller, 1974), 74-91% (Martin, 1974), and 65-70% (Dawson et al., 1975). Mechanical deboning of fish racks, after conventional filleting, results in yields in the range of 25-30% (Miyauchi and Steinberg, 1970), 30% (King, 1972), 27-35% (Crawford et al., 1972b), and 50% (Dawson et al., 1975) based on weight of the whole fish. Mechanical deboning of fish increases the amount of edible flesh recovered by 25 to 50%. The great variation in yields is due to large differences among species related to relative mass and anatomical features (Crawford et al., 1972b), (King, 1972). The belt pressure also affects yields. At normal tension the yield based on whole weight was 46.4-47.4% and at the light tension the yield was 32.5-34.1% (Miyauchi et al., 1975). The yield and quality were higher when the flesh was prepared from fish at the rigor mortis stage or immediately after resolution (Blackwood, 1974).

Proximate Composition

Proximate composition of the fish flesh is altered by mechanical deboning. The moisture content of machine separated flesh is slightly higher than the moisture content of hand separated flesh (Webb et al., 1976), (Babbit et al.,

1974), (Crawford et al., 1972a). The protein content of mechanically deboned meat is either less than (Babbit et al., 1976) or the same as (Webb et al., 1976) hand separated fish fillets. Also reported are significantly higher quantities of sarcoplasmic and nonprotein nitrogen and lower levels of collagen and connective tissue in the machine separated meat (Webb et al., 1976). The shear action of mechanical deboning near the skin causes increased levels of fat in mechanically deboned meat (Webb et al., 1976), (Babbit et al., 1974), (Crawford et al., 1972a). The ash content is reported to be the same or slightly higher in machine separated meat as compared to hand separated meat (Webb et al., 1976), (Chant et al., 1977).

Bones

The presence of small bones in the mechanically deboned fish flesh may prevent acceptance of the product. The frequency of occurance of bone particle depends largely on the size of the holes in which the minced muscle is passed. Long pieces of bone may be forced through the holes by shear forces (Patashnik et al., 1974). The bones may be pliable or hard depending on the adjustment of the equipment and the species. Removal of bones or an additional processing step in which the bones are softened may be necessary to develop acceptable products from mechanically deboned meat.

Color

The color of the cooked flesh of some of the less popular species is often grey or pinkish-grey (Iredale, 1975) and thus may prevent the acceptance of a product. The lighter color of minced flesh can be obtained by extensive washing to remove blood pigments or blending with suitable white flours (King, 1974). The undesirable grey color can be diluted by mixing fillet scraps into the product or using selective cuts from the frames when deboning (King, 1974). Tomato paste or paprika could help make a suitable pink or red colored product.

Lipid Oxidation

Mechanism of Oxidation

Fish products are subject to deterioration caused by microbial spoilage and oxidation. At temperatures less than $-7^{o}C$ microbial activity is essentially stopped, causing oxidative rancidity to be the main cause of product degradation (Bremmer et al., 1976). Labuza (1971) defined rancidity as the development of off-flavors which make the food unacceptable to a consumer at the market level. The "flavors" which develop are a direct result of the reaction between oxygen and unsaturated fatty acids which may or may not be a part of triglycerides or phospholipids. The site of oxidation in muscle tissue is usually the protein bound phospholipid fraction not readily extracted with fat solvents (Younathan and Watts, 1959).

The mechanism of oxidation is described by dividing the reaction into three parts: initiation, propagation, and termination (Labuza, 1971), (Karel, 1974), (Schultz et al., 1962). Karel (1974) describes the initiation step as the formation of a free radical (R·) which can be described as:

$$RH + O_2 \text{ ------ } R\cdot + OH\cdot$$

During the propagation step there is a rapid increase in the rate of oxygen absorption as the first free radical combines with molecular oxygen to form a peroxy radical (ROO·). Non-oxidized unsaturated fatty acids and the peroxy radical then combine to form new free radicals and hydroperoxides in the propagation step.

$$R\cdot + O_2 \text{ ------ } ROO\cdot$$

$$ROO\cdot + RH \text{ ------ } ROOH + R\cdot$$

These hydroperoxides are the first relatively stable intermediates; however they can be thermally or catalytically decomposed leading to the formation of low molecular weight carbonyls and other breakdown products. These new radicals are responsible for the taste and flavor of rancidity (Schultz et al., 1962).

The termination of the chain occurs with the formation of non-free radical products.

$$R\cdot + R\cdot \text{ ------ } RR$$

$$ROO\cdot + ROO\cdot \text{ ------ } ROOR + O_2$$

$$R\cdot + ROO\cdot \text{ ------ } ROOR$$

Lipids in Fish Muscle

There is a high proportion of unsaturated fatty acids in fish lipid, especially in the phospholipid and proteolipid fractions. Seasonal variation of fat content and fatty acid composition is largely due to food availability, sexual cycle, and maturity. Minimum fat levels occur in the late winter and the maximum in the summer (Stansby, 1973), (Dewitt, 1963). In many fish the highest degree of unsaturation occurs in the winter, followed by an abrupt decrease in unsaturation after spawning in spring and an increase slowly through a summer of intensive feeding (Dewitt, 1963), (Gruger et al., 1964). Location of the catch affects the degree of unsaturation and may be a reflection of the food available (Dewitt, 1963). Old fish tend to have more highly unsaturated long chain fatty acids than young fish (Gruger et al., 1964).

Sensitivity of lipids to rancidity changes are greater in the winter and early spring than in the summer and fall (Castell and Maclean, 1964a). There is little difference in fat content between male and female except that during the spawning season the lipids of the female may be slightly more susceptable to oxidation (Hansen, 1964).

The fat distribution varies throughout the body of an individual fish. There is a high percentage of fat in the tail end (Damberg, 1963), (Castell and Maclean, 1964a), (Labuza, 1971), and in the belly flap (Labuza, 1971), (Stansby, 1973) and the dark muscle is higher in fat than light

muscle (Zisper and Watts, 1961), (Castell and Maclean, 1964a), (Orthoefer, 1974), (Patashnik et al., 1973). The flesh from the tail end, belly flap and dark muscle is more susceptible to oxidation than muscle tissues from other locations in the body. Depending on how a fish is trimmed or prepared for deboning the fat content and susceptibility to oxidation of the deboned flesh may vary.

Prooxidants in Muscle Tissue

Several substances have been recognized as prooxidants for fish lipid oxidation. Heme pigments in fish flesh are believed to act as biocatalysts promoting oxidative rancidity (Zisper and Watts, 1961), (Schultz et al., 1962), (Froning and Johnson, 1973), (Dyer, 1974), (Kunsman and Field, 1976), (Deng et al., 1977). When the heavily pigmented lateral lines were removed from fish fillets and refrigerated they were subject to a greater degree of oxidation than less pigmented samples (Watts, 1961). Heat and acid treatments of fish will denature catalase and peroxidase resulting in exposure of heme groups and increases in nonenzymatic oxidation (Eriksson et al., 1971). Heme pigments and characteristics of the lipid of the fish can greatly affect the flavor.

The heme content of fish, especially in the heavily pigmented portion of the flesh, is considerably higher than the heme content in beef and poultry. Ocean mullet dark flesh contained 57.3 ppm iron as compared to 26 ppm iron in beef; indicating why fish may be more susceptible to

oxidation than beef (Fisher and Deng, 1977).

Mechanical deboning further increases the amount of heme pigment in the flesh primarily due to the addition of heme pigments from bone marrow (Kuneman and Field, 1976), (Froning and Johnson, 1973). Several mechanical washings of deboned meat with 3-7 parts water for every one part meat, helps to remove these heme pigments, fats, and water soluble components and increases the stability of the meat (King, 1972), (Kudo et al., 1973), (Miyauchi et al., 1975), (Patashnik et al., 1976). Heme pigments can be removed by centrifugation but the operation is quite expensive (Froning and Johnson, 1973). Washing of the minced flesh also improves color (Miyauchi et al., 1975), (Okado et al., 1973).

To a lesser extene myoglobin, another heme pigment, will encourage oxidation (Dyer, 1974), (Kuneman and Field, 1976). Labuza (1971) indicated that trace metals may be responsible for primary initiation of oxidation. During mechanical deboning, trace metals from the backbone may be incorporated into the meat which appears to decrease stability (Babbit et al., 1974), (Dyer, 1974). Other factors which may accelerate oxidation include light, irradiation, heavy metal salts, moisture, lipoxidase, and anything that may inactivate antioxidants (Schultz et al., 1962).

Antioxidants in Muscle Tissue

The development of oxidation may be inhibited by the addition of an antioxidant to the food system. Antioxidant action may be due to the donation of hydrogen or an electron

which reacts with free radicals to form inert products terminating the chain reaction mechanisms (Schultz et al., 1962). Phenolic antioxidants may aid the termination of polymerization by the addition of the lipid to the aromatic ring of the antioxidant or the formation of a complex between the lipid and the aromatic ring (Schultz et al., 1962). Microorganisms, which decompose hydroperoxides, may be useful in inhibiting reactions (Alford et al., 1971). Actively growing bacteria suppress the development of rancidity in fish muscle, but the mere presence of bacteria, even in large numbers, is not sufficient to inhibit rancidity development (Castell and Maclean, 1964b). Physical conditions such as low temperatures, removal of oxygen from the package, or holding the moisture content below a critical level will also inhibit oxidation. Increased levels of tocopherol in the diet of fish may slow the development of rancidity and reduce fishy flavors in the final product (Castell and Maclean, 1964a), (Gibson et al., 1977).

Activity of antioxidants is greatly enhanced in the presence of synergists such as certain acids and phospholipids. The antioxidant donates a hydrogen to the unsaturated fat which is, in turn, reactivated by a hydrogen from the acid or phospholipid, thereby continuing to have an antioxidant effect (Schultz et al., 1962). Synergists also have the ability to chelate trace metal thus reducing their catalytic effect.

Use of Antioxidants

Antioxidants may be added to the food system by first dissolving in a fat, oil, or water or by direct addition without a carrier. Painting, dipping, spraying, injecting, and pumping are other methods which have been used to apply antioxidants (Bentz et al., 1952). However, they are most effective when incorporated in the fat phase and uniformly distributed throughout the food system in the early stages of processing (Stuckey, 1956). Introduction at a later stage will not normally achieve maximum protection (U.S. Army, 1975).

Applications of various antioxidants to meat and other food systems have been investigated by several researchers. Butylated hydroxyanisole (BHA), an antioxidant stable to heat and mild alkali, has proven to be effective in a number of foods (Dyer et al., 1950). Kraybill et al. (1949) found BHA to be very effective in animal fats and Lehman and Watts (1950) reported that BHA was quite effective in an aqueous lard system. Marion and Forsythe (1962) were able to lengthen storage life of turkey with BHA, but other researchers were not able to stabilize fish products using it (U.S. Army, 1975), (Miyauchi et al., 1975), Sweet (1973) found that BHA and butylated hydroxytoluene (BHT) both retarded rancidity when used separately in trout but not in salmon. A combination of BHA and BHT increased storage stability of minced fish much better than BHA alone (Teeny and Miyauchi, 1972), (Babbit, 1972).

Other antioxidants have been added to meat systems with varying degree of success. Propyl gallate (PG), helped to control oxidation in turkey (Jacobson and Koehler, 1970) and Tenox II^{R} (a commercial preparation containing BHA, PG, and citric acid) helps to increase storage stability of chicken (Moerck and Ball, 1974). Development of rancidity in fish products has been modestly controlled with PG in smoked buffalofish stored at -02^{o}C (Greig, 1965). Propyl gallate was quite effective when added to buffalofish stored at -18^{o}C to -21^{o}C (Greig, 1965) and trout stored at 4-5^{o}C (Sweet, 1973). The development of rancidity was controlled with propyl gallate in fish portions stored in blocks at -15^{o}C to -18^{o}C but the antioxidant contributed a chemical taste to the product (Greig et al., 1967). The addition of nordihydroguaiaretic acid (NDGA) to ground buffalofish stored at -18^{o}C to -21^{o}C helped to retard development of rancid flavors as did the addition of Tenox II^{R} (Greig, 1965). The stability of salmon and trout stored at refrigeration temperatures was improved with the addition of tertiarybutylhydroquinone (TBHQ), (Sweet, 1973). Addition of TBHQ or ethylenediaminetetraacetic acid, (EDTA), along with ascorbic acid helped to curtail the development of rancidity in fishery products (Deng et al., 1977). Sweet (1977) found the stability of fatty fish was best controlled with the addition of TBHQ or BHA with EDTA or citric acid to the fish product. Sodium erythorbate was also successful in retarding oxidation and flavor deterioration in mechanically deboned

fish flesh (Iredale and York, 1977). A one minute dip of the racks, prior to deboning, in ascorbic and citric acids, Kena^{R} (Calgon), and NaEDTA with a post deboning addition of ascorbic and citric acids, Kena and NaEDTA, was an effective treatment used on deboned flounder racks (Molidina et al., 1977).

Citric acid, phosphoric acid, triethyl phosphate, and lecithin exhibit synergistic effects which increase the effectiveness of many antioxidants (Kraybill et al., 1949). Webb (1975) determined that an ice glaze of citric acid and water helped to improve stability of fish patties. Ascorbic acid is an active synergist when added directly with other antioxidants to fish (Tarr, 1948) and cooked meats (Tims and Watts, 1958). Greig et al. (1967) found ascorbic acid was an effective antioxidant when used alone in ground fish and portions stored in blocks at -18 ± 2^{o}C. The addition of ascorbic acid increased the shelf life of commercially prepared fish products from 4-6 months to 10-12 months (Greig et al., 1967).

Other Treatments for Controlling Oxidation

Heat Treatment

Severe heat treatment may have an antioxidant effect in cooked meat systems due to considerable destruction of heme pigments during prolonged heating and further loss of heme pigments which occur during subsequent storage. Destruction of the heme pigments appear to be related to their

function as a catalyst in lipid oxidation. Suppression of lipid oxidation may be due to the formation of an antioxidant in the meat during prolonged cooking. Addition of overcooked meat to raw meat was effective in preventing lipid oxidation in the new batch of meat (Watts, 1961). Heat treatment of tuna scrap, to be used for mink feed, helped reduce the development of rancidity, and heat treatment in combination with antioxidant was found to be even more effective (Sinnhuber and Yu, 1958).

Salt

The addition of NaCl to minced and comminuted meats causes solubilization of the muscle proteins and extraction of myofibrillar proteins, increasing the cohesiveness and binding ability of the muscle. The texture of several minced fish samples improved and became firmer with the addition of salt (Teeny and Miyauchi, 1972), (Cobb et al., 1974), (Miyauchi et al., 1975), (Patashnik et al., 1976) and in the absence of salt, textural strength of minced fish was weak and crumbly (Lee and Toledo, 1976). Moderate amounts of salt will improve the texture of minced fish although the effect will vary somewhat from species to species (Keay and Hardy, 1974b).

Addition of salt increased moisture retention in sausage (Swift and Ellis, 1957) and decreased cooking losses in restructured pork (Schwartz and Mandigo, 1976), in chicken (Farr and May, 1970), in beef (Moore et al., 1976) and in irradiated pork (Shults et al., 1976). Less drip was noted

in fish treated with salt than in control fish (Wierbicki et al., 1957). The hydrating effect of salt is due to the increased electrostatic repulsions between protein charges. Crosslinkages or polymerization between protein molecules limits the degree of moisture retention.

Although salt helps to improve texture and moisture retention, its effects are not all beneficial. Salt will encourage the development of oxidative rancidity and will decrease storage stability of fresh and frozen products. Other additives are necessary to offset the prooxidative activity of salt.

Phosphates

In the last thirty years much research has been carried out in an attempt to evaluate the effect of phosphates in a meat system. In order of increasing effectiveness, pyrophosphate, tripolyphosphate, heptaphosphate, and hexametaphosphate were determined to be effective as synergistic antioxidants (Watts, 1950). The antioxidant activity of phosphates may be due to the ability of phosphate to sequester metals. This may explain why the addition of phosphate helped to inhibit oxidation in fish (Zipser and Watts, 1961), (Ramsey, 1963), and in chicken (Younathan and Watts, 1959), (Farr and May, 1970). A combination of phosphate and ascorbic acid will prolong the protection of fats in food systems (Lehman and Watts, 1950), (Tims and Watts, 1958). Dipping lean fish fillets in a solution of tripolyphosphate and sodium ascorbate effectively inhibited the

development of rancid flavors (Watts, 1969). Phosphate may not always have an antioxidant effect, however, since the addition of sodium tripolyphosphate to ground cisco stored at -18 ± 2°C did not improve storage stability (Greig et al., 1967).

The pH of meat is increased by the addition of basic phosphates which may result in undesirable flavors when the pH is raised too much (Ramsey, 1963). More importantly, the pH change causes an increase in water absorption and retention in the food (Martin, 1974). Drip resulting from freeze-thaw cycles in chicken (Mountney and Arganosa, 1963) and beef (Morse, 1955) was greatly reduced with the use of phosphate. The improved water holding capacity due to the phosphates increased juiciness in comminuted meats (Tims and Watts, 1958), chicken (May et al., 1963), and restructured pork (Schwartz and Mandigo, 1974). Lower cooking losses were observed in ham and beef (Morse, 1955), fish (Dawson et al., 1975), chicken (Farr and May, 1970), (Froning, 1966), irradiated pork (Shults et al., 1976), and restructured pork (Schwartz and Mandigo, 1976) with addition of phosphates.

Other benefits of phosphate include texture improvements. The addition of 0.5 to 1.0% phosphate to chicken improved texture but at 2.0% added phosphate the texture was rubbery (Froning, 1966). Tensile strength and cohesion of meat was improved in sausage with added phosphate (Swift and Ellis, 1957).

Film Packaging

Moisture loss accelerates the development of oxidative rancidity, thus edible film coatings will prevent moisture losses thereby inhibiting oxidation. Lard was used as a protective film on frozen meats and did provide some protection against oxidation (Hiner et al., 1951). Moisture losses were decreased and skin darkening was minimized in chicken coated with an acetylated monoglyceride film (Dawson et al., 1963). Fresh fish were successfully preserved by spraying or immersing in acetylated monoglycerides and stored at 1-4^{o}C (Stemmler, 1974).

The temperature of application, duration of dip, and thickness of the film coating will affect the effectiveness of the film treatment. The amount of monoglyceride coating applied to chicken varied inversely with the solution temperature and immersion time (Dawson et al., 1963). Longer dip times at higher temperatures resulted in less protection of the chicken. Longer immersion times caused greater increases in the surface temperature and may have resulted in physical and chemical changes in the skin, thereby damaging the water binding mechanisms. Shorter dip times and lower temperatures may form films which are more likely to control oxidation.

Vacuum Packaging

Limiting the amount of oxygen available will decrease the rate of oxidation in meat products. Packaging a food in an atmosphere of carbon dioxide or nitrogen will effectively

prevent rancidity development but carbon dioxide will cause off-flavors (Tarr, 1948). Addition of glucose and glucose oxidase will also remove available oxygen from a packaging system which will oxidize hydrogen to water thereby eliminating available oxygen, but these packaging procedures are expensive (Karel, 1974).

Vacuum packaging will also retard oxidative changes without development of off-flavors. Storage life of beef and lamb (Bremmer et al., 1976) and fish (Deng et al., 1977) was increased by vacuum packaging. The quality of silver salmon steaks was improved and the improvement was noted even with fluctuating storage temperatures (Yu et al., 1973). Vacuum packaging alone is not a totally adequate means of protection, however, oxygen removal in combination with an antioxidant treatment should be more beneficial minimizing oxidation.

2-Thiobarbituric Acid (TBA) Tests

The 2-thiobarbituric acid (TBA) test has been used as a relative measure of rancid flavor development in several food products. The peroxide test is not as useful as the TBA test to detect oxidative rancidity (Tims and Watts, 1958). The fat solvents used for the peroxide test may not extract the oxidation products of highly unsaturated protein bound phospholipids causing the test to be less useful. Sensory evaluation scores correlate well with TBA values obtained for beef (Bengt et al., 1972), (Haymon et al., 1976), pork (Turner et al., 1972), and fish (Zisper and

Watts, 1962), (Yu and Sinnhuber, 1957), (Maclean and Castell, 1964), (Greig, 1965), (Greig et al., 1967). TBA values obtained for some fish products did not correlate with sensory evaluation scores (Botta and Shaw, 1976), (Deng et al., 1977).

The lack of correlation between TBA values and sensory scores may be due to the interference of carbohydrates from breading in the fish products. Turner et al. (1955) showed that the addition of 1% sucrose to a fish sample caused a 71% increase in TBA values, while the addition of 1% glucose and 1% starch did not change values. The presence of some carbohydrates causes the formation of a yellow interfering compound which affects TBA values (Caldwell and Grogg, 1955). The small amounts of naturally occurring sugars in beef and pork did not interfere with the TBA tests (Turner et al., 1955).

EXPERIMENTAL PROCEDURE

Source and Preparation of Fish Samples

Source of Fish

The first samples of mullet (Catostomus catostomus) used to determine the effect of various antioxidants in summer harvested fish were obtained from the northern edge of Lake Michigan in Eponfette Bay. All other fish samples were Catostomus commersoni obtained from Point Au Gres in the Saginaw Bay, Lake Huron. The fish were either held in ice or in live-holding tanks until they were transported to laboratory facilities. The samples were placed into stainless steel lugs, packed with ice, and transported to Michigan State University by truck. The fish were held in ice at $4^{o}C$ until the following morning. All fish were headed and gutted, washed, split, and mechanically deboned using a Bibun deboning machine (SDX13, 5 mm holes, Bibun Co., Fukuyama Hiroshima, Japan). The minced fish flesh was held at $4^{o}C$ until further treatment.

Application of Antioxidants

In the first experiment, four treatment groups were used to assess the effectiveness of four commerical preparations in preventing the development of oxidative rancidity in minced fish flesh. The four preparations were:

1) Tenox A^{R} - 40% butylated hydroxyanisole, 8% citric acid, 52% propylene glycol; Eastman Chemical Products Inc., Kingsport, Tennessee

2) Tenox II^{R} - 20% butylated hydroxyanisole, 6% propyl gallate, 4% citric acid, 70% propylene glycol; Eastman Chemical Products Inc., Kingsport, Tennessee

3) Tenox PG^{R} - 100% propyl gallate; Eastman Chemical Products Inc., Kingsport, Tennessee

4) Freez-Gard^{R} (FP88E) - NaCl, Na tripolyphosphate, Na erythorbate; Calgon Corp., Pittsburgh, Pennsylvania

The amount of Tenox A and Tenox II added was 0.02% of the weight of the fat present in each fish sample. Tenox PG, being a dry compound, was first made into solution at a ratio of 36% Tenox PG to 64% propylene glycol, then, based on the percentage of fat in the fish, 0.01% of the solution was added to the third treatment group. A calibrated syringe was used to apply the antioxidant treatments to insure accuracy. For the fourth treatment group, twenty-four grams of Freez-Gard were dissolved in 100 ml of distilled water, then 4.53 grams of this solution were added for every 100 grams of fish. This resulted in an addition of 0.18% Freez-Gard based upon the weight of the entire sample. A control sample with 4.53 grams of distilled water per 100 grams of meat was prepared to determine if the added water affected the quality of the fish flesh. All antioxidant preparations were mixed into the flesh using a KitchenAid Food Preparer (Model K5-A, Hobart Mfg., Troy, Ohio) with a paddle attachment at medium speed for three minutes.

Packaging and Storage

All of the treated samples and untreated control were packed in polyethylene whirl-pac bags (Scientific Products, Evanston, Illinois) and stored at -18°C. Samples were removed from the freezer after 1, 3, 6 and 12 months to chemically evaluate the development of rancidity using the TBA test. This procedure was used to prepare samples for evaluation from both summer and winter fish catches.

Pattie Formulation

A portion of the minced fish flesh was treated with 0.18% Freez-Gard, based on the weight of the whole sample, and mixed in a paddle-type mixer (Model 1/2BB, John E. Smith and Sons, Buffalo, New York) for three minutes. Another sample was prepared by mixing a binder preparation containing 58.7% ice, 11.8% sugar, 11.8% salt, 11.8% corn oil, 3.5% monosodium glutamate, 1.8% Na tripolyphosphate, 0.5% ascorbate, and 0.1% nitrite with the meat. Ten grams of the binder preparation were added for each kilogram of meat and mixed by hand for three minutes. A third portion of fish was treated with both Freez-Gard and binder. Meat samples were stuffed into fibrous casings, 88 mm diameter, using a water pressure powered piston sausage stuffer (E-Z Pak, E.F. Zuber Engineering and Sales Co., Minneapolis, Minnesota). The stuffed casings were labeled and frozen overnite at -18°C. The following day the frozen minced fish was tempered at room temperature for ten minutes and sliced into approximately 60 g patties using a table meat saw (Quikut

Saw, Model AH2661, Wells Manufacturing Co., Three Rivers, Michigan). Patties were stored in cryovac bags (Standard Gauge, Type S-503, W.R. Grace and Co., Simpsonville, South Carolina) at -18°C until further treatment.

Filming and Breading

Some of the patties with Freez-Gard and some without Freez-Gard were dipped for one second into Myvacet distilled acetylated monoglycerides (Type 700-A, Eastman Kodak Co., Rochester, New York) at 110°C to form a protective edible film. The film increased the weight of the patties by approximately 8.2%. All patties were breaded using a batter prepared from 4.5 cups of Drake's Batter Mix (Drake's Batter Mix Co., Grass Lake, Michigan), 4 cups of water, 60 grams of salt, and 80 g of Old Bays seasoning. Individual patties were dipped into batter, allowed to drain for five seconds, and then frozen. The weight of the patties increased by approximately 22.3% due to breading.

Precooking

Half of the patties treated with Freez-Gard, binder, Freez-Gard and binder, monoglyceride, or untreated were precooked in corn oil for thirty seconds at 177°C in a Hotpoint duothermostat fryer (Model HK3 General Electric, Chicago Hts., Illinois). Patties were stored at -18°C in cryovac bags until packaged.

Packaging and Storage of Patties

All precooked and raw patties were placed in polyethylene mylar laminated IKD Super All Vac pouches

(International Kenfield Distributing Co., Parkridge, Illinois) and sealed using a vacuum sealer (Model C-14), International Kenfield Distributing Co., Parkridge, Illinois). Patties from each treatment group were packed either in air and other patties were vacuum packed. Samples for chemical tests were packaged individually and patties used for sensory evaluation were packaged in units of eight, with wax paper separating each pattie. Samples were stored at -18^{o}C and removed from the freezer at 1, 3, 6 and 12 months of storage for evaluation.

Chemical Tests

Preparation of Samples

After each batch of fish was deboned a portion of the minced flesh was set aside for proximate analyses. The samples were frozen in cryovac bags at -18^{o}C in small units and thawed at room temperature preceding each analysis. The calcium, fat, moisture, and protein content of each minced flesh sample was determined within two weeks of deboning.

To measure the development of oxidative rancidity one bag of minced fish from each antioxidant treatment in the first experiment and one pattie from each treatment in the second were analyzed at the end of each storage period. Samples were thawed as quickly as possible in cool water. The minced fish samples were placed in plastic weighing boats and mixed using a micro-spatula to insure a homogenous sample. To prepare the patties for analysis, as much

breading as possible was removed and the monoglyceride film was removed from the appropriate samples. These samples were also mixed in plastic weighing dishes to insure homogeneity.

Calcium Content

Calcium content was determined by the method of Steagall (1965).

After the sample was thawed 10.0 grams of meat were transferred into a 200 ml graduated Erlenmeyer flask and 15 ml of water, 15 ml of 2N hydrochloric acid and several glass beads were added. The mixture was boiled until digested (about 20 minutes), cooled, made up to a volume of 200 ml with water, and mixed. The digest was filtered and 20 ml of the filtrate was transferred to a 250 ml beaker. The pH of the filtrate was adjusted to 12.5 with KOH-KCN and hydroxy naphthol blue indicator, Mallinckroft 5630, was added. A 0.02M ethylenediamine-tetraacetic acid solution was used to titrate to a blue-green endpoint. Percent calcium was determined by multiplying the ml of titrate used by 0.8. Four replications were performed on each sample.

Fat Content

The fat content was determined using the Goldfisch extraction method (A.O.A.C. 1975, 24.0056).

Four to six grams of unfrozen fish flesh were placed in a previously dried and weighed moisture dish, accurately weighed and dried in a forced air oven at 105^{o}C overnite.

The aluminum dish and sample were placed in an alundum vessel and clipped into the Goldfisch apparatus. Approximately 30 ml of anhydrous diethyl ether were poured into a Goldfisch beaker which had been previously oven dried, cooled, and weighed. The beaker was connected to the apparatus and the ether was allowed to boil for four hours. After the extraction was complete the ether was boiled off and reclaimed. The Goldfisch beaker containing the fat was then oven dried at 105°C for one hour, cooled for 30 minutes in a dessicator and weighed. The percent fat was calculated as the grams of fat extracted for each one hundred grams of meat. There were four replicates for each sample.

Moisture Content

The A.O.A.C. (1975, 25.0036) procedure for determining moisture was used for all fish samples. Three to five grams of thawed fish flesh were accurately weighed into an aluminum dish that had been previously dried at 105°C for two hours, cooled in a dessicator, and weighed. Each dried sample was cooled in a dessicator for 30 minutes and weighed. The percentage moisture was calculated per one hundred grams of meat. Four replicates were run for each sample.

Protein Content

Protein content was determined following the micro-Kjeldahl nitrogen determination method (A.O.A.C. 1975, 23.009).

One gram sodium or potassium sulfate, 3 to 5 ml of concentrated sulfuric acid, one ml of 10% copper sulfate

solution, a few boiling beads, and 0.5 grams of fish flesh were added to an Aminco Kjeldahl digestion flask. The mixture was heated on a digestion rack under a hood until the boiling mixture was clear. The digestion continued for thirty minutes longer. The flasks were allowed to cool and 25 ml of deionized water were added to each.

Next a sample was placed on the distillation apparatus and 125 ml wide mouth beaker containing 10 ml of 2% boric acid and one drop of brom cresol green indicator was put under the condenser stem. Five ml of 50% NaOH for each ml of concentrated sulfuric acid used in the original digestion were added into the sample. The distillation proceeded until 20 ml of distillate were collected. The ammonia-boric acid solution was titrated to the brom cresol green endpoint with .1833N sulfuric acid. Blanks without the meat sample were run and the titration values were subtracted from the titration values of the samples.

The percent protein was calculated using the following formula:

$$\% \text{ protein} = \frac{(.1833)(\text{ml of sulfuric acid})(14)(6.25)(100)}{\text{weight of sample}}$$

Four replicates were run on each sample.

2-Thiobarbituric Acid (TBA) Test

The TBA values were determined using a method of Tarladgis et al., (1960).

Four 10 gram portions of the minced meat or patties were homogenized with 50 ml of distilled water at medium speed for one minute in a Virtis homogenizer (Model 6-105-AF, Virtis Co., Gardiner, New York). The homogenized mixture was transferred into a 500 ml distilling flask with the aid of 47.5 ml of distilled water. The pH was lowered to 1.5 using 2.5 ml of 4N HCl. Two drops of Dow Corning Antifoam A (Dow Corning Corp., Midland, Michigan) and a few glass beads were added to the mixture. The flask was connected to a distilling unit consisting of a 30.5 cm long distilling column connected to the condensor with a bending elbow, and a 50 ml graduated cylinder acted as a receiver. After boiling began, the first 50 ml of distillate were collected.

Two 5 ml portions of the distillate were pipetted and transferred to screw top test tubes. Then five ml of 0.02M thiobarbituric acid (Eastman Organic Chemical, Rochester, New York) in 95% redistilled glacial acetic acid were added and the tubes were capped, mixed and heated in a boiling water bath for 35 minutes. After cooling in cold water for ten minutes the absorbance was determined at 538 nm against a reagent blank in which five ml of distilled water were used in place of the distillate.

The TBA number was calculated by multiplying the mean absorbance by 7.8, a distillation constant (Tarladgis et al., 1960). The TBA value was reported as mg TBA reactive substance per 1000 grams of meat.

Sensory Evaluation

Sample Preparation

Samples were removed from the freezer and immediately fried in corn oil at 177°C for five minutes using a Hotpoint duothermostat fryer. The patties were allowed to drain after cooking to remove excess cooking oil. Both raw and precooked samples were handled by the same procedure. Fish patties were cut into quarters, placed in shallow pans, covered with aluminum foil, and held in a convection oven at 98°C.

Score Card

An open-ended hedonic scale was used for sensory evaluation of fish patties. Appearance, flavor, texture, and general acceptability of patties were rated on a scale of one to seven. A score of seven indicated very desirable appearance, flavor, or acceptability; a score of one indicated that the pattie was undesirable and four represented neutrality. For texture, a score of near four was the most desirable with values greater than four indicating rubberiness and less than four indicating crumbliness (Appendix A). Panelists were presented four treated samples, water, and crackers at each serving.

Panel Members

Panelists were selected at random from staff and students in the Food Science and Human Nutrition Dept. Each taste panel included 20 panelists and was performed at the end of each storage period for patties made of fish caught in the summer.

Statistical Analyses

Statistical analyses were performed by a Michigan State University computer program identified as Michigan State University Agricultural Experiment Station STAT Program AOV and run on a Control Data Corporation (CDC) 6500 computer. Correlation coefficients between TBA values and flavor scores were calculated using a Canon, Canola F-20P electronic printing calculator (Canon, USA, Inc., Elmhurst, Illinois).

RESULTS AND DISCUSSION

Freshwater mullet were mechanically deboned, treated with antioxidants, packaged, and held frozen for periods of up to 12 months. Other samples of freshwater mullet were mechanically deboned, formed into patties, treated, packaged, and held frozen for periods of up to ten months. Calcium, fat, moisture, and protein contents were determined on all fresh mechanically deboned meat samples. At each test period samples were thawed and prepared for chemical evaluation to determine changes in lipid oxidation. Some of the fish patties were evaluated to determine sensory characteristics.

Proximate Composition and Yields

Proximate composition and yields of mechanically deboned fish are reported in Table 1. The proposed maximum percent calcium content for mechanically deboned meat is 0.75% and for mechanically deboned meat for processing, is 1.00% (anon., 1976). The calcium content of all samples of mechanically deboned fish were well below the proposed maximum levels. Differences were detected between calcium content of each harvest, which were probably due to the adjustment on the mechanical deboning equipment.

Table 1. Proximate composition and yields of mechanically deboned freshwater mullet caught at various times of the year.

Date of Catch[1]	Calcium %	Fat %	Moisture %	Solids %	Protein %	Yield %
			EXPERIMENT 1			
8/26/75 Summer	0.110	5.91	76.81	23.19	17.28	47.3
12/04/75 Winter	0.130	2.03	79.55	20.45	16.18	39.6
			EXPERIMENT 2			
2/24/76 Winter	-	2.27	79.69	20.31	14.69	-
7/01/76 Summer	0.063	2.22	80.46	19.54	16.88	46.6
7/15/76 Summer	0.092	3.19	78.70	21.30	17.06	51.0

[1] The 8/26/75 harvest was Catostomus catostomus, all other harvests were Catostomus commersoni.

A substantial variation was found between the fat content of various harvests. The mechanically deboned flesh from fish harvested in the summer, (August, Lake Michigan), season had the highest fat content. The fish harvested from Lake Huron yielded mechanically deboned flesh with similar fat contents, with those harvested in December having slightly lower fat levels than those harvested in July. Food availability, weather, and time of harvest may have contributed to the variability. Generally the percentage fat appears to increase as summer feeding continues. The harvest of 8/26/75, the sample with the highest fat content, was a different species and the only sample taken from Lake Michigan indicating that location and species may affect fat content.

There is an inverse relationship between the fat and moisture content; as the fat content increased the moisture content decreased. This relationship is common in most meat systems. There is also an inverse relationship between the solids content and the moisture content.

The protein content of the mechanically deboned meat remained fairly constant. Mechanically deboned meat from the fish harvested in February had the lowest protein content. This decrease toward the end of the winter may be due to low food availability and high energy demands necessary to survive the winter.

Minced flesh yields varied and tended to be higher in fish harvested in July and August. Lower moisture content

appeared to favor higher yields. Some of the lower yields may have been due to moisture loss; lower initial moisture content would help prevent losses. Adjustment of the equipment and belt tension also greatly affected yields. Different individuals helped head, gut and split the fish, at each deboning session, thus variations in technique may have contributed to variation in yield.

2-Thiobarbituric Acid (TBA) Test - Antioxidant Treatments

The mean TBA numbers are reported in Table 2. TBA values for mechanical deboned meat from summer harvested fish are illustrated graphically in Figure 1 and from winter harvested fish, in Figure 2.

The mechanically deboned meat made from summer harvested fish developed higher TBA values than that from winter harvested fish, indicating a greater degree of oxidation. The most probable reason for the higher TBA values of the summer harvested flesh is that the fat content of the mechanically deboned meat was substantially higher than from the winter harvested fish.

As storage time increased, the TBA values increased regardless of treatment. Longer periods of time favored development of the products of oxidation.

Results from both the summer harvest and the winter harvest reveal that Freez-Gard was the most effective treatment of those evaluated for preventing oxidation. After each storage period, the Freez-Gard treated flesh had significantly lower TBA values, (Table 2), than controls and

Table 2. Mean TBA values[1] for mechanically deboned freshwater mullet harvested in the summer and winter, treated with and without antioxidants; and stored at -18°C for up to 12 months.

Storage Time (Months)	Treatment					
	Tenox A	Tenox II	Tenox PG	Freez-Gard	Control w/Water	Control w/o Water
Summer (August)						
1	0.6^{b}	0.9^{bcd}	1.1^{cd}	0.3^{a}	1.2^{d}	0.7^{bc}
3	1.5^{b}	1.9^{b}	2.2^{bc}	0.4^{a}	3.3^{c}	1.6^{b}
6	3.2^{b}	3.3^{b}	3.7^{b}	0.6^{a}	3.9^{b}	2.9^{b}
12	9.0^{bc}	6.7^{b}	10.5^{c}	1.5^{a}	10.1^{bc}	9.0^{bc}
Winter (December)						
1	0.4^{b}	0.3^{b}	0.3^{b}	0.1^{a}	0.5^{b}	0.4^{b}
3	0.5^{b}	0.5^{bc}	0.6^{bc}	0.1^{a}	0.8^{c}	0.8^{c}
6	1.5^{b}	1.6^{b}	2.1^{bc}	0.4^{a}	1.7^{bc}	2.5^{c}
12	3.2^{b}	3.4^{b}	3.5^{b}	0.6^{a}	2.6^{b}	3.6^{b}

[1]The TBA values of the mechanically deboned meat at day "0" was 0.3 for both summer and winter harvests. All TBA values with the same superscript are not significantly different at the 5% level using Duncan's Multiple Range Test for each storage period.

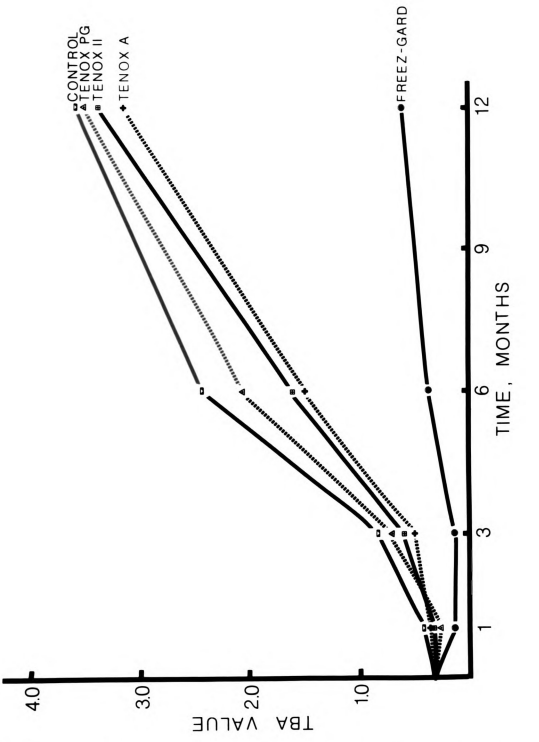

Figure 1. TBA values of mechanically deboned freshwater mullet harvested in the summer, treated with and without antioxidants, and stored at -18°C for up to 12 months.

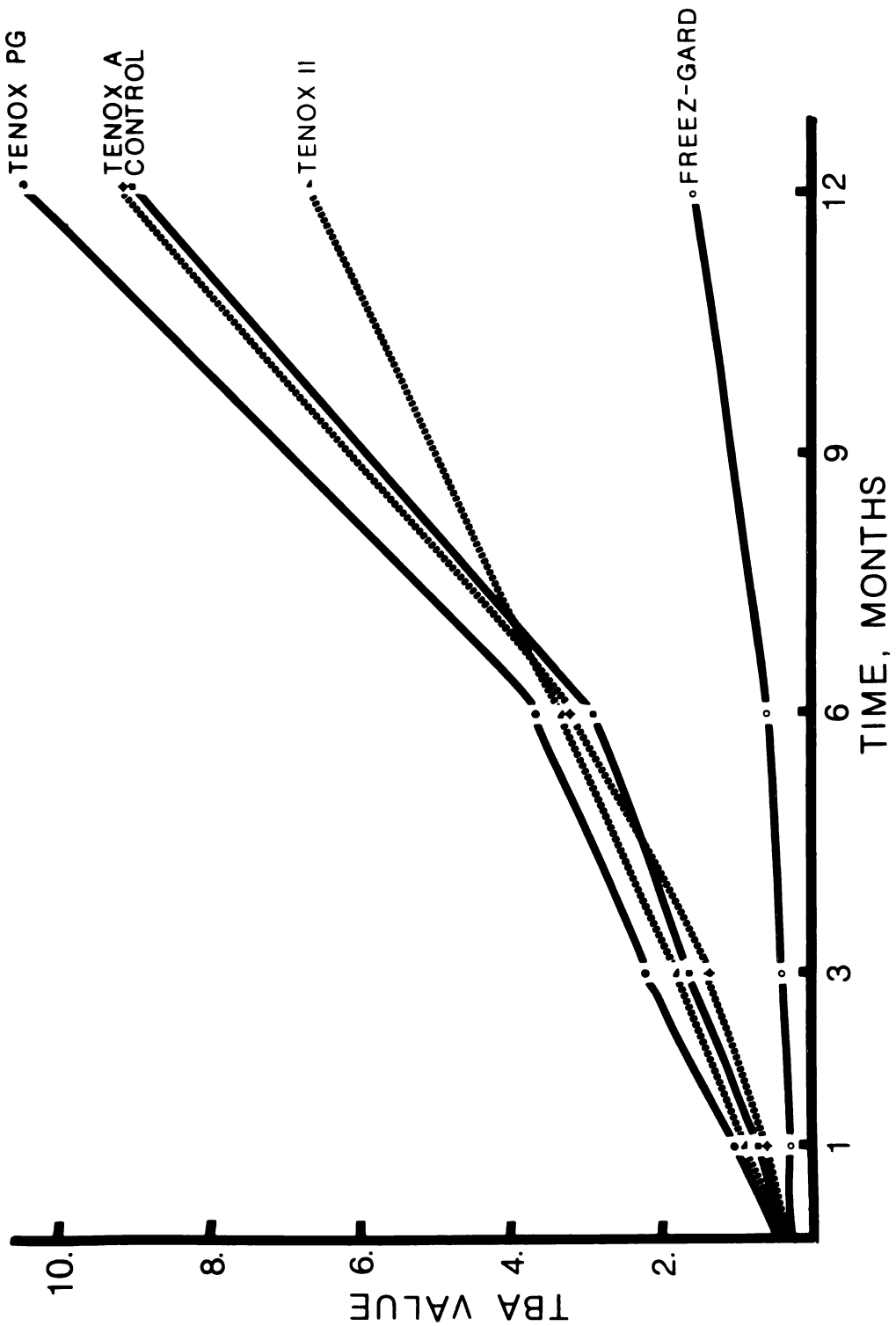

Figure 2. TBA values of mechanically deboned freshwater mullet harvested in the winter, treated with and without antioxidants, and stored at -18°C for up to 12 months.

flesh treated with other antioxidants. As storage time increased, the effectiveness of Freez-Gard became more apparent (Fig. 1,2). The superiority of Freez-Gard is probably due to the presence of tripolyphosphate and erythorbate. The ability of the tripolyphosphate to sequester metals and increase water retention and water absorption, contributes to the antioxidant qualities of the compound. Sodium erythorbate is able to interfere with heme catalyzed lipid oxidation. The antioxidant activity is not due to the salt, which is a prooxidant. Salt was incorporated into Freez-Gard to improve texture, flavor, and water binding capacity.

Water was used to add Freez-Gard to the fish flesh, so samples with added water but no Freez-Gard served as a second control to evaluate the effect of the water. The added water had no antioxidant effect. The TBA values for the controls with and without water were not significantly different except after one and three months of storage of the summer harvested fish, (Table 2). The control with water had higher TBA values than the summer controls without water. The addition of water may have a prooxidant effect, or mixing may incorporate oxygen in the sample thus hastening increases in TBA values.

The mean TBA values for mechanically deboned freshwater mullet harvested in the summer and treated with Tenox A, Tenox II, and Tenox PG were not significantly different than the mean TBA values of control samples without water

(Table 2). After one month of storage the TBA values of the flesh treated with Tenox PG were significantly higher than TBA values of Tenox A treated flesh and after 12 months the TBA values of the Tenox PG treated flesh were significantly higher than the TBA values of the flesh treated with Tenox II. Addition of phenolic antioxidants did not prevent a change in TBA values in the summer harvested fish flesh.

Statistical analyses of the mean TBA values of mechanically deboned fish harvested in the winter indicated that there were no significant differences among the flesh samples treated with Tenox A, Tenox II, and Tenox PG. After three months of storage the TBA values of the Tenox A treated flesh were significantly lower than the values of the control without water and after six months storage the values of flesh treated with Tenox II and Tenox A were significantly lower than those of the controls without water. Tenox II and Tenox PG were not effective antioxidants in the winter harvested fish, however, Tenox A did show promise as a short term antioxidant.

Analysis of variance of the mean TBA values is reported in Table 3. Greatest significant differences were attributed to storage time (months), with the largest F value, followed by season (summer vs. winter). All two way interactions showed similar levels of significance, and these interactions can be noted in Fig. 1 & 2 where plotted lines cross one another.

Table 3. Analyses of variance of TBA values of mechanically deboned freshwater mullet treated with various antioxidants and stored at -18°C for up to 12 months

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
Season	1	6.973	1099.01	<0.0005
Month (in storage)	3	7.710	1215.01	<0.0005
Treatment[1]	5	2.380	375.20	<0.0005
Season Month	3	0.123	19.46	<0.0005
Season Tmt.	5	0.058	9.08	<0.0005
Month Tmt.	15	0.043	6.74	<0.0005
Season Month Tmt.	15	0.014	2.14	0.011

[1]Treatment: Tenox A, Tenox II, Tenox PG, Freez-Gard, Control w/water, Control w/o water.

Addition of phenolic antioxidants does not seem to prevent or slow down the development of oxidative rancidity. A few of the fish samples with phenolic antioxidants had lower TBA values than controls at the end of a storage period, however, there were no consistent trends to justify the use of the phenolic antioxidants in mechanically deboned fish flesh. These antioxidants may not have been effective due to the incorporation of oxygen into the fish samples during the mixing operation. Watts (1961) abandoned the use of phenolic antioxidants, BHA, NDGA, and PG, as a means of stabilizing fish, since the compounds do not have sufficient water solubility to allow for adequate distribution throughout the fish flesh.

2-Thiobarbituric Acid (TBA) Test - Fish Patties

Based on the results of the first trial, Freez-Gard was selected as the antioxidant treatment to be used in the formulation of the fish patties. The second experiment was to confirm the effectiveness of Freez-Gard, and to evaluate the effects of a binder, monoglyceride film, precooking, and vacuum packaging on product quality and stability.

Mean TBA values for patties made from mechanically deboned freshwater mullet harvested in the summer are reported in Table 4 and from fish harvested in the winter are reported in Table 5. Mean TBA values for patties made of mullet harvested in the summer with similar treatments are summarized in Table 6 and from fish harvested in the winter are

Table 4. Mean TBA values[1] for patties made from mechanically deboned freshwater mullet harvested in the summer and stored at -18°C for up to 10 months

Sample Treatments	Storage Time (months)			
	1	3	6	10
Raw, Control:				
Air	1.4	3.6	4.1	3.9
Vacuum	1.0	2.8	4.7	3.9
Binder-Air	0.7	1.3	2.6	2.0
Binder-Vac	0.5	1.8	1.4	2.4
Film-Air	0.9	1.5	1.4	1.6
Film-Vac	0.5	0.9	1.0	0.8
Precooked, Control:				
Air	1.4	2.1	2.5	2.5
Vacuum	0.7	0.7	0.7	0.4
Binder-Air	0.7	1.2	1.4	2.1
Binder-Vac	0.4	0.6	0.8	0.6
Film-Air	0.9	2.1	2.7	2.6
Film-Vac	0.7	0.7	0.9	1.0
Raw, Freez-Gard:				
Air	0.3	0.7	0.9	1.5
Vacuum	0.3	0.5	0.7	0.4
Binder-Air	0.3	0.6	0.8	1.2
Binder-Vac	0.3	0.4	0.3	0.4
Film-Air	0.3	0.4	0.5	0.5
Film-Vac	0.3	0.4	0.4	0.5
Precooked, Freez-Gard:				
Air	0.4	0.6	1.0	1.5
Vacuum	0.4	0.5	0.7	0.6
Binder-Air	0.4	0.5	0.4	0.9
Binder-Vac	0.4	0.4	0.3	0.4
Film-Air	0.4	0.5	0.3	0.5
Film-Vac	0.3	0.4	0.3	0.3

[1]The TBA value of the mechanically deboned meat at day "0" was 0.1.

Table 5. Mean TBA values[1] for patties made from mechanically deboned freshwater mullet harvested in the winter and stored at -18°C for up to 10 months

Sample Treatments	Storage Time (months)			
	1	3	6	10
Raw, Control:				
Air	1.7	5.7	8.2	7.1
Vacuum	0.8	2.5	5.7	6.1
Binder-Air	0.7	2.2	4.4	6.4
Binder-Vac	0.5	1.4	2.6	4.1
Film-Air	0.9	3.3	3.6	3.9
Film-Vac	0.8	1.3	3.1	2.5
Precooked, Control:				
Air	1.4	3.5	6.5	7.6
Vacuum	0.9	2.3	5.3	7.3
Binder-Air	0.3	1.1	3.5	5.3
Binder-Vac	0.4	1.2	2.5	4.0
Film-Air	1.5	4.3	5.5	6.8
Film-Vac	1.3	3.5	5.2	4.4
Raw, Freez-Gard:				
Air	0.3	1.6	4.4	5.1
Vacuum	0.3	1.0	2.3	3.4
Binder-Air	0.3	0.6	1.4	2.2
Binder-Vac	0.3	0.6	1.3	2.0
Film-Air	0.2	0.6	0.7	1.2
Film-Vac	0.2	0.4	1.3	1.1
Precooked, Freez-Gard:				
Air	0.3	0.7	3.5	5.3
Vacuum	0.3	0.7	2.2	3.8
Binder-Air	0.2	0.3	0.8	1.3
Binder-Vac	0.2	0.2	0.7	1.1
Film-Air	0.3	0.3	0.8	2.3
Film-Vac	0.3	0.4	1.3	2.8

[1]The TBA value of the mechanically deboned meat at day "0" was 0.4.

Table 6. Mean TBA values[1] for patties made from mechanically deboned freshwater mullet harvested in the summer and stored at -18°C for up to 10 months, summarized by treatments.

Sample Treatments	Storage Time (months)			
	1	3	6	10
No Freez-Gard	0.8	1.6	2.0	2.0
Freez-Gard	0.3	0.5	0.6	0.7
Raw	0.6	1.2	1.6	1.6
Precooked	0.6	0.9	1.0	1.1
No Treatment	0.7	1.4	1.9	1.8
Binder	0.5	0.9	1.0	1.3
Monoglyceride Film	0.5	0.9	0.9	0.9
No Vacuum	0.7	1.3	1.6	1.7
Vacuum	0.5	0.8	1.0	1.0

[1]The TBA value of the mechanically deboned meat at day "0" was 0.1.

summarized in Table 7. Statistical analyses of the data are reported in Table 8 and the Appendix B.

Mean TBA values for patties with the same treatment from mullet harvested in the winter are higher than those for patties from mullet harvested in the summer. The mechanically deboned meat from the winter harvest used to formulate patties may have been more susceptible to oxidation than the summer harvest because the flesh was held in casings in frozen storage for almost one month before the patties were formed. The patties from summer harvested fish were formed one day after stuffing into the casing. Dewitt (1963) and Gruger et al. (1964) found lipids of winter harvested fish were most susceptible to oxidation due to higher levels of unsaturation in the fish lipids.

Comparisons between summer and winter harvested fish in this experiment appears to contradict the results of the first experiment. The winter harvested flesh was must susceptible to oxidation and in the first experiment the summer harvested fish were most prone to oxidation. The summer harvested fish used to evaluate the effect of various antioxidants had the highest fat content of all the catches and these fish were a different species and the only group caught in Lake Michigan. The high fat content, species, and the different location of harvest probably affected the susceptibility of the fish lipids. The results of the experiment are not conclusive enough to establish in which season the fish lipids are most susceptible to oxidation, however, it is

Table 7. Mean TBA values[1] for patties made from mechanically deboned freshwater mullet harvested in the winter and stored at -18°C for up to 10 months, summarized by treatments.

Sample	Storage time (months)			
	1	3	6	10
No Freez-Gard	0.9	2.7	4.7	5.5
Freez-Gard	0.3	0.6	1.7	2.6
Raw	0.6	1.8	3.3	3.8
Precooked	0.6	1.5	3.2	4.3
No Treatment	0.8	1.0	4.8	5.7
Binder	0.4	1.0	2.2	3.3
Monoglyceride Film	0.7	1.8	2.7	3.1
No Vacuum	0.7	2.0	3.6	4.5
Vacuum	0.5	1.3	2.8	3.6

[1]The TBA value of the mechanically deboned meat at day "0" was 0.4.

Table 8. Analyses of variance of TBA values of patties made of mechanically deboned freshwater mullet with various treatments and stored at -18°C for up to 10 months. TBA values averaged over season and months in storage.

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
Cook[1]	1	0.689	6.34	0.012
Freez-Gard[2]	1	41.095	378.52	<0.0005
Binder[3]	2	4.890	45.04	<0.0005
Vacuum[4]	1	3.971	36.58	<0.0005
Cook FG	1	0.038	0.35	0.554
Cook Binder	2	1.168	10.76	<0.0005
Cook Vac	1	0.071	0.67	0.418
FG Binder	2	0.300	2.76	0.064
FG Vac	1	0.481	4.43	0.036
Binder Vac	2	0.147	1.35	0.259
Cook FG Binder	2	0.428	3.94	0.020
Cook FG Vac	1	0.156	1.43	0.232
Cook Binder Vac	2	0.011	0.10	0.905
FG Binder Vac	2	0.211	1.94	0.144
Cook FG Binder Vac	2	0.041	0.37	0.688

[1]Cook - raw, precook

[2]FG-No Freez-Gard, Freez-Gard

[3]Binder - no treatment, binder, monoglyceride film

[4]Vac - no vacuum, vacuum

believed that the lipids are more sensitive to rancidity changes in the winter and early spring (Castell and Maclean, 1964a).

As the length of the storage period increased to six months, the TBA values increased (See Table 4 & 5). Most of the TBA values for the patties after 12 months of storage were higher than the TBA values after six months storage, but a few of the TBA values decreased during the second half of storage. Most of the samples which had TBA values that decreased after 6 months were vacuum packed. Deng et al. (1977) and Nakayama and Yamamoto (1977) also detected a decrease in TBA values of some fish products stored for long periods.

There are several theories as to why TBA values would decrease over time. For oxidation to occur, oxygen is needed and after the available oxygen is used oxidation will cease. The TBA values of vacuum packed patties are more likely to decrease or stabilize because there is little oxygen available. Dent et al. (1977) postulated that the products of lipid hydrolysis, free fatty acids, may react with proteins in a manner that protected double bonds against oxidation. The decrease in TBA values may not be due to the suppression of oxidation but to the unavailability of TBA reactive malonaldehyde. Malonaldehyde has been found to react with amino groups of trout myosin (Botta and Richards, 1973). Cysteine, methionine, and the proteins in tuna have also been reported to bind malonaldehyde (Botta and

Richards, 1973). It is possible that over a period of storage, malonaldehyde becomes bound to the proteins of the fish patties thereby causing a decrease in TBA values.

Analysis of variance of TBA values of patties made from mechanically deboned freshwater mullet show that all main effects and most interactions were significant (Appendix B). To evaluate the effect of the various treatments, TBA values for patties with identical treatments for each storage period were averaged together. Analysis of variance for mean TBA values is recorded in Table 8. The magnitude of the F statistic was used to determine the relative significance of the main effect and interactions.

The F statistic for the Freez-Gard main effect was several times larger than any F statistic for the other main effects and interactions. As concluded in the first experiment, Freez-Gard is a very effective means of preventing the development of oxidative rancidity.

The addition of the binder preparation and the monoglyceride film helped to slow down the increase in the TBA numbers. The mean TBA values for the patties treated with the binder and film and the control are reported in Tables 6 and 7 and graphically illustrated in Figure 3.

There are several reasons why the binder preparation would have antioxidant properties. The mixture contains sodium tripolyphosphate, which is also an ingredient of Freez-Gard. The phosphate's ability to sequester metals and retain moisture may affect the antioxidant activity. The

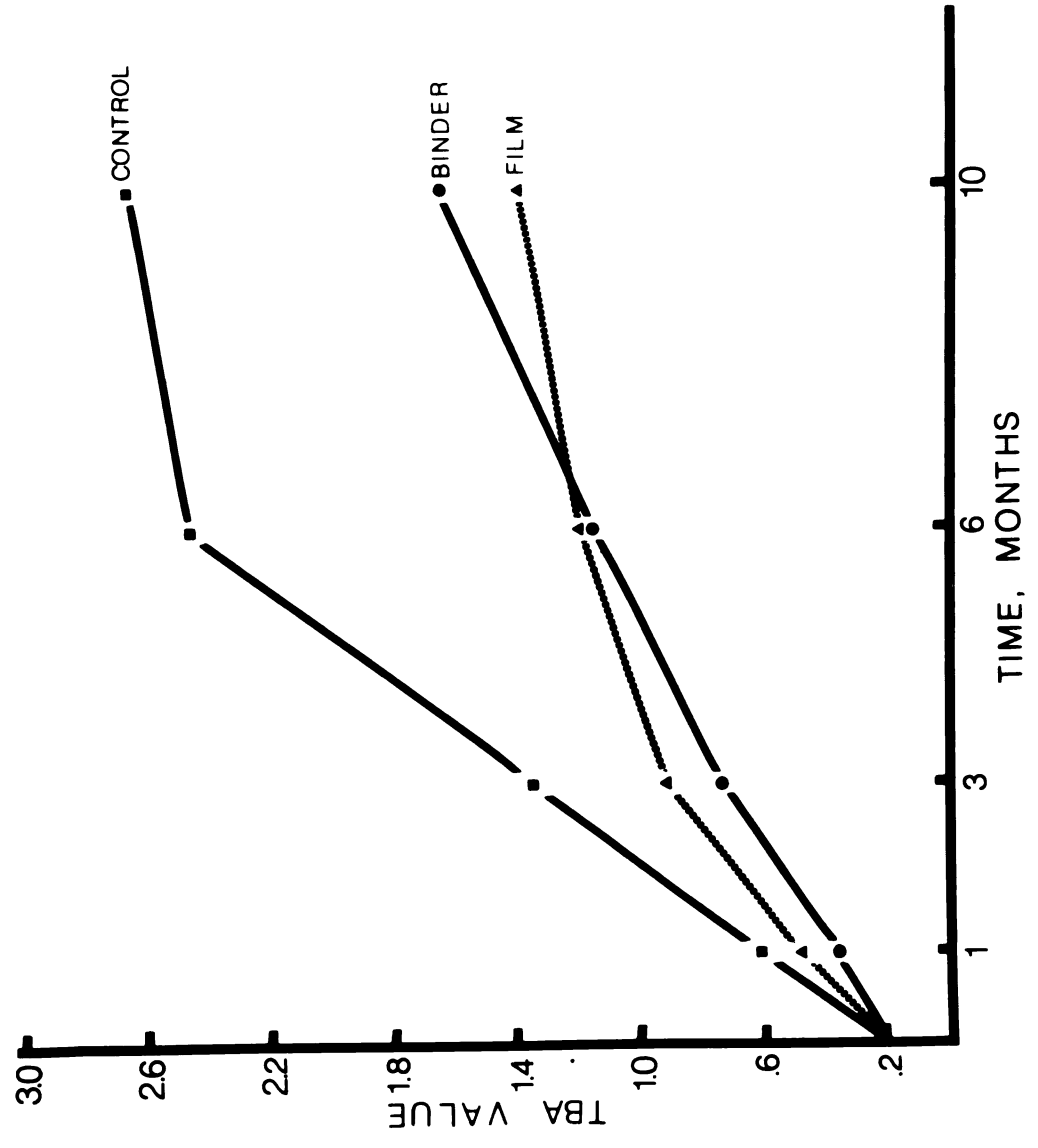

Figure 3. Mean TBA values of fish patties made from mechanically deboned freshwater mullet treated with a binder and a film stored at -18°C for up to 10 months.

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preparation contains ascorbate, which has synergistic characteristics and has been used in other fish products as a means of curtailing the development of oxidation (Tarr, 1948), (Greig et al., 1967). The nitrite present in the binder preparation may have some antioxidant quality. Cross and Zeigler (1965) reported that nitrite interferes with the oxidation of unsaturated lipids, possibly by deactivating hematin catalysts.

The monoglyceride film helped to retard the increase in TBA values. The film gives protection by preventing moisture losses, thereby inhibiting oxidation. The availability of oxygen is also decreased by coating the patties with the monoglyceride film.

Precooking the patties was a useful means of preventing oxidation, except when the patties were coated with a monoglyceride film. The mean TBA values for raw and precooked patties are reported in Tables 6 and 7 and graphically illustrated in Figure 4. Comparison of F statistics indicates that precooking is not as effective in preventing oxidation as the addition of Freez-Gard, binder or vacuum packaging. Other workers have reported that cooking or precooking is an effective means of slowing the development of oxidation. Keay and Hardy (1974a) found flash frying provided protection against oxidation and the U.S. Army (1975) found that frying lowered levels of oxidative changes.

Precooking may have an antioxidant effect for several reasons. Cooking causes destruction of the heme pigment thus

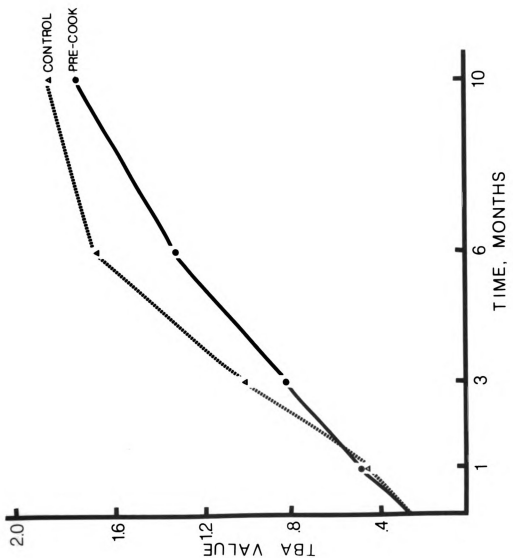

Figure 4. Mean TBA values of raw and precooked fish patties made from mechanically deboned freshwater mullet stored at -18°C for up to 10 months.

prevents the pigments from acting as catalysts in lipid oxidation. It is also possible that some type of antioxidant may be formed in the meat system during cooking by a mechanism not yet understood, or perhaps antioxidants from the cooking oil may have become incorporated in the patties during precooking.

A significant interaction was found between the cooking treatment and the addition of the binder and monoglyceride film. Precooking improved the stability of the control patties and the patties with the binder but decreased the stability of the patties coated with the film. During precooking the monoglyceride film melted and some of the breading flaked off from the patties. Melting the film decreased the ability of the film to prevent moisture loss and exclude oxygen.

Vacuum packing was found to be a very effective means of preventing the development of oxidative rancidity. The mean TBA values for vacuum packed and air packed patties are reported in Tables 6 and 7 and graphically presented in Figure 5. Removal of oxygen from the packaging system prevented or minimized the initiation step of the oxidation process. Without the continual formation of new free radicals in the initiation step, the oxidation process was slowed down considerably. Vacuum packing does not completely prevent oxidation, however, residual oxygen can initiate oxidation and the chain reaction effect in the propagation step will allow some oxidation to occur.

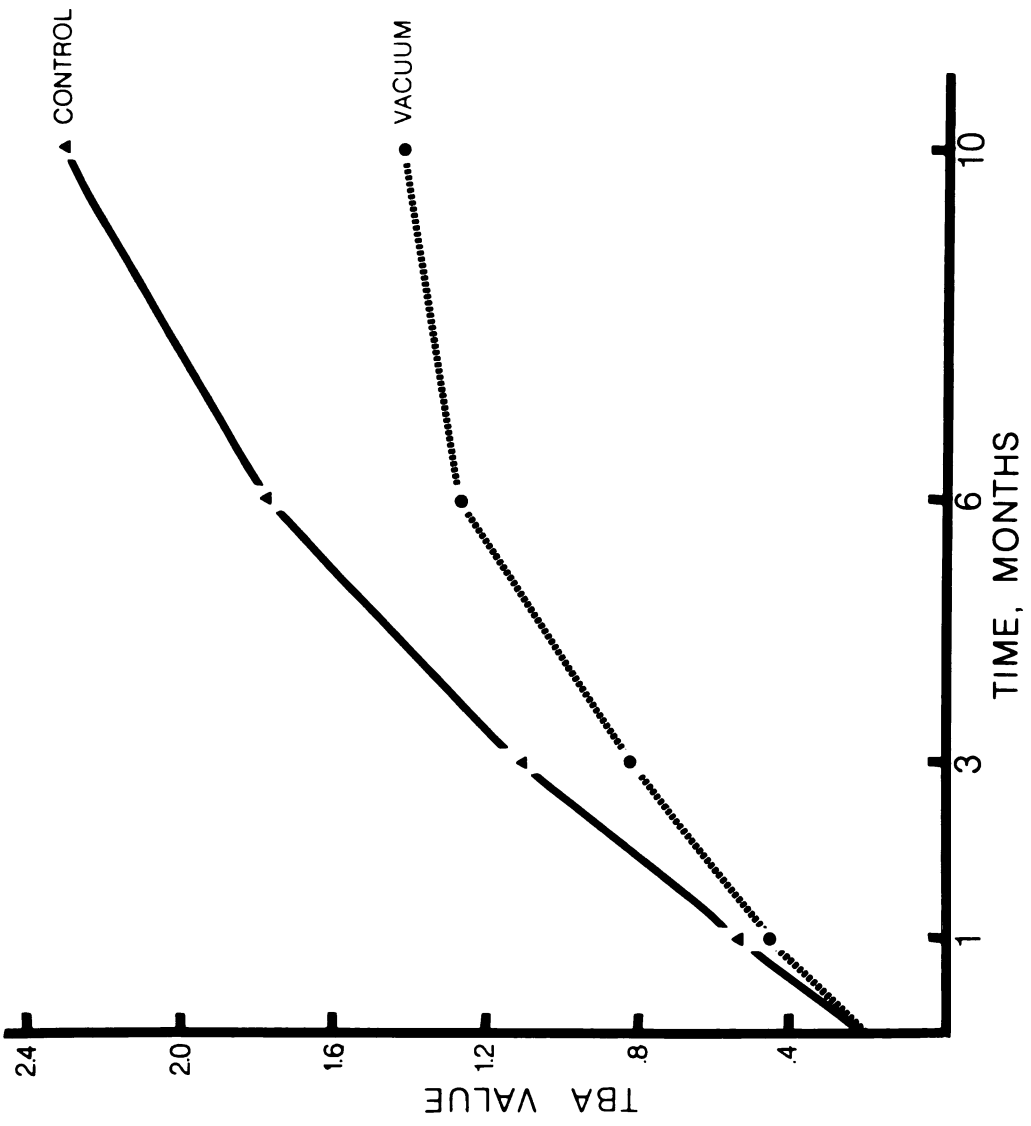

Figure 5. Mean TBA values of air packed and vacuum packed patties made from mechanically deboned freshwater mullet stored at -18°C for up to 10 months.

The final color of many of the TBA reactive solutions from patties were yellowish orange to orange instead of the usual TBA light pink to deep red color. Molidina et al. (1977) observed a yellowish color in samples of mechanically deboned flounder treated with ascorbic and citric acids, sodium polyphosphates, and Na_2EDTA. Further research demonstrated that the added chemicals alone were not responsible for the development of the yellow color (Molidina et al., 1977). The formation of the aberrant color may have been the result of a reaction between TBA reagents and substance(s) in the mechanically deboned flesh besides malonaldehyde. Caldwell and Grogg (1955) determined that the presence of some carbohydrate caused the formation of a yellow compound. Perhaps a component of the breading contributed to the yellow-orange color in the TBA final color. The affect of the yellow-orange color on the absorbance and the TBA values was not determined.

Sensory Evaluation

Fish patties were evaluated by a panel of twenty persons using a score card designed to record appearance, flavor, and general acceptability scores. A sample score card is shown in the Appendix A. The mean appearance, flavor, texture and general acceptability scores for all fish patties evaluated over a six month period are reported in Tables 9 and 10, and the statistical analyses of these data are shown in Tables 11 and 12. The mean appearance,

Table 9. Mean appearance and flavor scores for minced fish patties with and without Freez-Gard treated with a binder, monoglyceride film, or precooked and stored at -18°C for up to 6 months

Sample Treatments	Storage Time (months)		
	1	3	6
		APPEARANCE	
Control:			
Untreated	5.7	5.0	5.1
Binder	5.3	5.1	5.4
Film	3.7	4.4	3.4
Precook	5.4	5.5	4.9
Freez-Gard:			
Untreated	5.0	5.2	5.1
Binder	5.0	5.1	5.1
Film	3.1	4.3	3.6
Precook	5.1	4.9	5.4
		FLAVOR	
Control:			
Untreated	4.9	4.2	4.9
Binder	4.9	4.3	4.1
Film	3.9	3.5	3.7
Precook	5.2	4.3	4.1
Freez-Gard:			
Untreated	4.7	4.7	4.8
Binder	5.2	4.9	4.5
Film	3.3	4.6	3.7
Precook	4.5	4.6	5.0

Table 10. Mean texture and general acceptability scores for minced fish patties with and without Freez-Gard treated with a binder, monoglyceride film, or precooked and stored at -18°C for up to 6 months

Sample Treatments	Storage Time (months)		
	1	3	6
		TEXTURE	
Control:			
Untreated	4.4	4.3	4.0
Binder	4.5	4.4	3.9
Film	5.7	4.6	4.9
Precook	4.7	4.9	5.0
Freez-Gard:			
Untreated	4.5	4.3	5.0
Binder	4.6	4.0	4.8
Film	5.7	5.9	5.5
Precook	4.9	4.3	5.1
		GENERAL ACCEPTABILITY	
Control:			
Untreated	4.8	4.3	4.8
Binder	4.6	4.3	4.3
Film	3.8	3.5	3.4
Precook	4.9	4.5	4.1
Freez-Gard:			
Untreated	4.7	4.6	4.7
Binder	5.0	4.9	4.5
Film	2.9	4.5	3.6
Precook	4.5	4.3	4.8

Table 11. Analyses of variance of appearance and flavor scores for patties made from mechanically deboned freshwater mullet stored at -18°C for up to 6 months

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
		APPEARANCE		
Month	2	1.190	0.71	0.491
Freez-Gard[1]	1	3.008	1.80	0.180
Treatment[2]	3	64.539	38.61	<0.0005
Month FG	2	3.165	1.89	0.152
FG Tmt.	3	0.069	0.04	0.989
Month Tmt.	6	2.887	1.73	0.113
Month FG Tmt.	6	1.401	0.84	0.541
		FLAVOR		
Month	2	2.144	0.97	0.382
Freez-Gard[1]	1	6.075	2.74	0.099
Treatment[2]	3	23.497	10.58	<0.0005
Month FG	2	8.856	3.99	0.019
FG Tmt.	3	0.875	0.39	0.757
Month Tmt.	6	3.241	1.46	0.191
Month FG Tmt.	6	2.481	1.12	0.351

[1]FG - Freez-Gard, No Freez-Gard

[2]Tmt. - No treatment, Binder, Monoglyceride film, Precook

Table 12. Analyses of variance of texture and general acceptability scores for patties made from mechanically deboned freshwater mullet stored at -18°C for up to 6 months

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
		TEXTURE		
Month	2	3.194	1.80	0.167
Freez-Gard[1]	1	9.352	5.26	0.022
Treatment[2]	3	26.858	15.11	<0.0005
Month FG	2	4.415	2.48	0.085
FG Tmt.	3	2.481	1.60	0.189
Month Tmt.	6	1.033	0.58	0.746
Month FG Tmt.	6	3.070	1.73	0.113
		GENERAL ACCEPTABILITY		
Month	2	0.890	0.42	0.655
Freez-Gard[1]	1	2.852	1.36	0.245
Treatment[2]	3	28.330	13.48	<0.0005
Month FG	2	4.577	2.18	0.114
FG Tmt.	3	0.835	0.40	0.755
Month Tmt.	6	2.676	1.27	0.268
Month FG Tmt.	6	2.685	1.28	0.266

[1]FG - Freez-Gard, No Freez-Gard

[2]Tmt. - No treatment, Binder, Monoglyceride film, Precook.

flavor, texture, and general acceptability scores are graphically represented in Figures 6, 7, 8, and 9 respectively.

The appearance scores of the patties did not change significantly as storage time increased, and the addition of Freez-Gard did not significantly affect the appearance scores. The patties with the monoglyceride film had significantly lower appearance scores than the other patties, since the monoglyceride caused the breading to flake and peel off during the cooking operation (Table 9). The meat was exposed and became brown, very dry, and quite unappealing. Dipping the patties in the monoglyceride film detracted greatly from the appearance of the patties.

No significant differences were found between flavor scores of patties treated with Freez-Gard over six months of storage. The flavor scores of the control patties decreased significantly after three months of storage. The addition of Freez-Gard helped prevent a decrease in flavor scores over time. A significant difference between the flavor scores of the patties with the film and the other patties was found and the patties with the monoglyceride film were rated lowest (Table 9). Cooking without breading resulted in patties becoming dry, while also altering flavor. Panelists complained that the patties with the film were bland and dry. The use of Freez-Gard seemed to prevent flavor deterioration over time and the monoglyceride film had an undesirable effect on flavor.

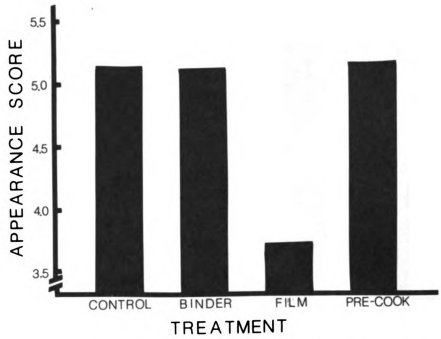

Figure 6. Mean appearance scores for fish patties made from mechanically deboned freshwater mullet precooked or treated with a binder or a film, and stored at -18°C for up to 6 months.

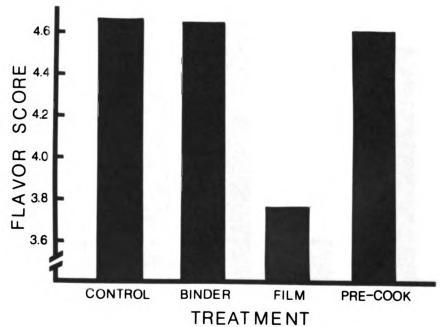

Figure 7. Mean flavor scores for fish patties made from mechanically deboned freshwater mullet precooked or treated with a binder, or a film, and stored at -18°C for up to 6 months.

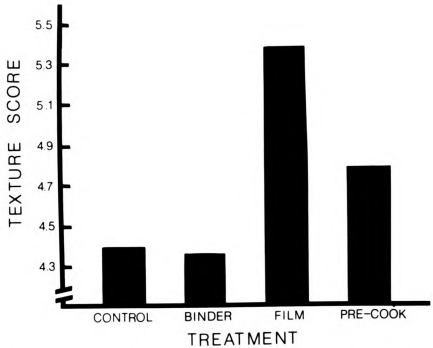

Figure 8. Mean texture scores for fish patties made from mechanically deboned freshwater mullet precooked or treated with a binder or a film, and stored at -18°C for up to 6 months.

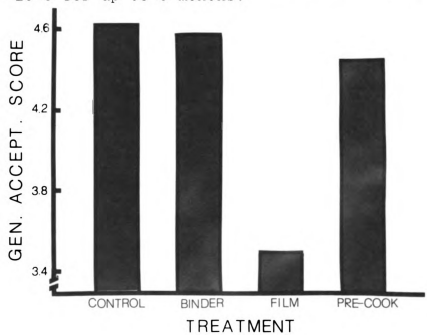

Figure 9. Mean general acceptability scores for fish patties made from mechanically deboned freshwater mullet precooked or treated with a binder or a film, and stored at -18°C for up to 6 months.

The mean texture scores of the patties with Freez-Gard were significantly more firm than the control patties. The salt in the Freez-Gard caused solubilization of muscle proteins and extraction of myofibrillar proteins, which increased the cohesiveness and binding capacity of the minced meat. The phosphates in the Freez-Gard probably also contributed to the increase in firmness. Although there was a difference detected between patties with Freez-Gard and the controls, panelists did not indicate whether the firmer texture was desirable or not.

Patties which were precooked had a significantly firmer texture than control patties and those treated with a binder (Table 10). This could be expected because cooking denatures sarcoplasmic and myofibrillar proteins and decreases water holding capacity thereby toughening the product. The patties with the monoglyceride film were the most firm, as the loss of breading during the cooking procedure caused the extreme moisture loss in the patties, due to the direct exposure to the hot cooking oil.

Nakayama and Yamamoto (1977) and Iredale and York (1977) found that fish products made from mechanically deboned flesh became more firm over extended periods of storage. The patties used for TBA analyses were observed to be more firm, as storage time increased. Some of the older samples were quite rubbery. Jarenbock and Liljemark (1975) believed formaldehyde and other lipid oxidation products formed during frozen storage, may cause a progressive

crosslinking of molecules with the protein filaments. This crosslinking would increase firmness. The loss of fluid, thaw drip, may also be related to increasing firmness in frozen storage. To minimize the fluid loss, starch may be added to the minced fish product (Cobb, 1974).

No significant differences were found in general acceptability scores as the length of storage time increased. The addition of Freez-Gard did not alter the general acceptability scores significantly. The patties with the monoglyceride film were rated significantly lower than the other patties (Table 10). The poor appearance, the dry, bland flavor, and the firm texture contributed to the low general acceptability scores.

The general acceptability scores generally reflected the flavor scores, the flavor of the patties being more important to the general acceptability than appearance and texture. Yu et al. (1973) found panelists relied heavily on flavor when evaluating the general acceptability of silver salmon steaks.

Correlation of TBA Numbers and Sensory Scores

Although sensory evaluations do not provide precise quantitative measurements of oxidative rancidity, they are widely accepted due to their sensitivity and reliability. The mean flavor scores were correlated with the mean TBA values to determine if any relationship existed between the subjective and objective results of the experiment. The

results are shown in Table 13.

Correlation coefficients for the fish patties varied irregularly between treatments. The TBA values were not always in agreement with sensory scores. High negative correlation coefficients were found for control patties which were precooked, treated with a binder, or film, and Freez-Gard patties treated with a binder. A lower negative correlation coefficient was present for the untreated control pattie. Decreasing sensory scores were accompanied by an increase in TBA values for some of the patties.

High positive correlation coefficients were obtained for the precooked Freez-Gard patties and the Freez-Gard patties without further treatment. A lower correlation coefficient was obtained for the Freez-Gard patties with a film. As TBA values increased, so did the sensory scores of some patties. The TBA values of the patties treated with Freez-Gard did not increase as much as TBA values of the control samples. The positive correlation coefficients indicate the changes in the flavor of the Freez-Gard treated patties were not related to the development of oxidative rancidity or else people prefer rancid flavors.

The overall coefficient for all the patties was very low (-0.08). There was little overall agreement between the subjective and objective results of this experiment. The TBA values did not correlate with flavor scores for mechanically deboned flounder (Molidina, 1977), however other researchers have obtained good correlation between TBA numbers and

Table 13. Correlation coefficients between TBA numbers and flavor scores of patties made from mechanically deboned fish

Sample Treatments	Correlation Coefficients
Control:	
Untreated	-0.34
Binder	-0.91
Film	-0.87
Precook	-0.98
Freez-Gard:	
Untreated	0.76
Binder	-0.98
Film	0.29
Precook	0.99
Overall	-0.08

sensory scores (Andersson and Danielson, 1961), (Greig, 1965), (Greig et al., 1967). The off-flavors in the mechanically deboned mullet may be due to factors other than rancidity development. Many panelists indicated the presence of fishy and oily flavors. Addition of tomato paste, potato flour, corn meal, or spices may be necessary to dilute or mask flavors inherent in the particular species of fish.

The relatively low TBA values of the patties made of summer harvested fish may have contributed to the low overall correlation coefficient. It takes TBA values of 2 to 4 or more to influence flavor; none of the patties made from summer harvested fish treated with Freez-Gard had TBA values greater than two. Rancid flavors may not have been developed enough for the panel to detect them.

SUMMARY

The development of oxidative rancidity was evaluated for mechanically deboned fish treated with various antioxidants. The development of off-flavors and muscle lipid oxidation in raw and precooked fish patties following different antioxidant treatments was evaluated under air and vacuum packed conditions. Sensory evaluation were conducted to determine effectiveness of the treatments. Oxidation of muscle lipid was evaluated using the 2-thiobarbituric acid (TBA test). The following observations were obtained:

1. Mechanically deboned meat treated with Freez-Gard showed significantly lower TBA values than did meat treated with Tenox A, Tenox II, and Tenox PG, and controls. Fish patties treated with Freez-Gard had significantly lower TBA values than patties without Freez-Gard.
2. Precooking improved the stability of the control patties and the patties treated with a binder but decreased the stability of patties coated with the monoglyceride film.
3. The TBA values of patties treated with the binder preparation were lower than TBA values of the control samples. The binder preparation is a

useful means of minimizing oxidative rancidity.

4. Storage time could be lengthened using the monoglyceride film treatment. However sensory evaluations indicated that poor texture and appearance prevented the film treatment from being a practical means of preventing rancidity.
5. Vacuum packaging was found to be more effective than packaging in air for deterring the development of rancidity. Antioxidant treatment in combination with vacuum packaging would be an even more effective means of increasing storage stability.
6. Poor correlation between sensory scores and TBA values existed in the fish patties made of mechanically deboned fish. Other factors besides the development of rancidity may contribute to the acceptance of the fish patties.

The best procedure to follow to prevent the development of oxidative rancidity in fish patties made from mechanically deboned freshwater mullet would be to treat with:

1. Freez-Gard
2. The Binder Preparation
3. Precook
4. Vacuum Package

All of the above treatments, or any combination thereof, would help to lengthen the storage stability of

the fish patties.

APPENDICES

APPENDIX A

Table A. Minced fish patties sensory evaluation sheet.

Name_____ Date_____

Evaluate appearance, flavor, texture, and general acceptability of each sample according to appropriate scale.

Appearance, flavor and general acceptability		Texture	
7	Like very much	7	Very dense/firm
6	Like moderately	6	Mod. dense/firm
5	Like slightly	5	Sl. dense/firm
4	Neither like nor dislike	4	Satisfactory
3	Dislike slightly	3	Slightly soft
2	Dislike moderately	2	Moderately soft
1	Dislike very much	1	Too soft

Select an adjective to describe flavor from the following list:

Fish, Salty, Spicy, Rancid, Oily: if other please specify.

Record the results in the table below.

RESULTS:

Sample number	Appearance score	Flavor score	Texture score	Description of flavor	Gen. accep.-tability

APPENDIX B

Table B. Analyses of variance of TBA values of patties made from mechanically deboned freshwater mullet with various treatments and stored at -18°C for up to 10 months.

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
Season	1	13.843	1369.02	<0.0005
Month	3	12.476	1233.91	<0.0005
Cook[1]	1	0.689	68.10	<0.0005
Freez-Gard[2]	1	41.095	4064.25	<0.0005
Binder[3]	2	4.890	483.57	<0.0005
Vacuum[4]	1	3.971	392.74	<0.0005
Season Month	3	3.441	340.27	<0.0005
Season Cook	1	0.197	19.51	<0.0005
Season FG	1	0.117	11.58	0.001
Season Binder	2	0.504	49.88	<0.0005
Season Vac	1	0.800	79.12	<0.0005
Month Cook	3	0.163	16.11	<0.0005
Month FG	3	0.224	22.18	<0.0005
Month Binder	6	0.234	23.17	<0.0005
Month Vac	3	0.169	16.72	<0.0005
Cook FG	1	0.038	3.77	0.053
Cook Binder	2	1.168	115.55	<0.0005
Cook Vac	1	0.071	7.04	0.008
FG Binder	2	0.299	29.61	<0.0005
FG Vac	1	0.481	47.62	<0.0005
Binder Vac	2	0.147	14.53	<0.0005
Season Month Cook	3	0.139	13.72	<0.0005
Season Month FG	3	0.142	14.04	<0.0005
Season Month Binder	6	0.020	1.97	0.067
Season Month Vac	3	0.094	9.25	<0.0005
Season Cook FG	1	0.823	81.40	<0.0005
Season Cook Binder	2	0.187	18.52	<0.0005
Season Cook Vac	1	0.442	43.68	<0.0005
Season FG Binder	2	0.127	12.52	<0.0005
Season FG Vac	1	0.002	0.23	0.633

APPENDIX B (cont.)

Source of Variation	d.f.	Mean Square	F Statistic	Approx. Signif. Prob. of F Stat.
Season Binder Vac	2	0.026	2.59	0.076
Month Cook FG	3	0.027	2.68	0.046
Month Cook Binder	6	0.027	2.67	0.015
Month Cook Vac	3	0.029	2.86	0.037
Month FG Binder	6	0.149	14.76	<0.0005
Month FG Vac	3	0.043	4.29	0.005
Month Binder Vac	6	0,033	3.26	0.004
Cook FG Binder	2	0,428	42,33	<0.0005
Cook FG Vac	1	0.156	15.39	<0.0005
FG Binder Vac	2	0.211	20.87	<0.0005
Season Month Cook FG	3	0.036	3.58	0.014
Season Month Cook Binder	6	0.023	2.27	0.035
Season Month Cook Vac	3	0.034	3.33	0.019
Season Month FG Binder	6	0.038	3.74	0.001
Season Month FG Vac	3	0.013	1.27	0.282
Season Month Binder Vac	6	0.064	6.30	<0.0005
Season Cook FG Binder	2	0.202	20.02	<0.0005
Season Cook FG Vac	1	0.626	61.87	<0.0005
Season Cook Binder Vac	2	0.053	5.23	0.006
Season FG Binder Vac	2	0.024	2.33	0.099
Month Cook FG Binder	6	0.045	4.47	<0.0005
Month Cook FG Vac	3	0.044	4.40	0.005
Month Cook Binder Vac	6	0.021	2.06	0.056
Month FG Binder Vac	6	0.059	5.85	<0.0005
Cook FG Binder Vac	2	0.041	4.02	0.018

[1]Cook - Raw, Precook.

[2]FG - No Freez-Gard, Freez-Gard.

[3]Binder - No treatment, Binder, Monoglyceride film.

[4]Vac - No vacuum, Vacuum.

LIST OF REFERENCES

REFERENCES

Alford, J.A., J.L. Smith and H.D. Lilly. 1971. Relationship of microbial activity to changes in lipids of foods. J. Appl. Bact. 34:133.

Andersson, K. and C.E. Danielson. 1961. Storage changes in frozen fish: A comparison of objective and subjective tests. Food Technol. 15:55.

Anonymous. 1976. Food regulations: Interim rules for deboned and low temperature rendered meat. Food Processing 37(7):8.

A.O.A.C. 1975. "Official Methods of Analysis", 12th Ed. Association of Official Agricultural Chemists, Washington, D.C.

Babbit, J.K. 1972. Minced flesh research and development at OSU Seafoods Laboratory. In Press. Oak Brook Seminar Mechanical Recovery and Utilization of Fish Flesh. Oak Brook, IL. Sept. 21-22.

Bentz, R.W., T.J. O'Grady and S.B. Wright. 1952. Antioxidants and food preservation. Food Technol. 6:302.

Blackwood, C.M. 1974. Summary of deliberation on minced fish at FAO Technical Conference in Tokyo - 1973. In Proc. 2nd Technical Seminar on the Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13.

Botta, J.R. and D.H. Shaw. 1976. Chemical and sensory analysis of Roundnose Grenadier (Coryphaenoides rupestris) stored in ice. J. Food Sci. 41:1285.

Bremner, H.A., A.L. Ford, J.J. MacFarlane, D. Ratcliff and N.T. Russel. 1976. Meat with high linoleic acid content: Oxidative changes during frozen storage. J. Food Sci. 41:757.

Caldwell, E.F. and B. Grogg. 1955. Application of the thiobarbituric acid test to cereal and baked products. Food Technol. 9:185.

Carver, J.H. and F.J. King. 1971. Fish scrap offers high quality protein. Food Eng. 43(1):75.

Castel, C.H. and J. Maclean. 1964a. Rancidity in lean fish muscle. II. Anatomical and seasonal variations. J. Fish. Res. Bd. Canada 21:1361.

Castel, C.H. and J. Maclean. 1964b. Rancidity: Lean fish muscle. III. The inhibiting effect of bacterial activity. J. Fish. Res. Bd. Canada 21:1371.

Chant, J.C., L. Day, R.A. Field, W.G. Kruggel and Y.O. Chang. 1977. Composition and palatability of mechanically deboned meat and mechanically separated tissue. J. Food Sci. 42:306.

Christie, W.J. 1974. Changes in the fish species composition of the Great Lakes. J. Fish Res. Bd. Canada 31:827.

Cobb, B.F. and C. Yeh. 1974. Improvement of texture, taste, and shelf-life on minced Atlantic Croakers. In Proc. 2nd Technical Seminar on the Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13.

Crawford, D.L., D.K. Law and J.K. Babbit. 1972a. Nutritional characteristics of marine food fish carcass waste and machine separated flesh. J. Agr. Food Chem. 20:1048.

Crawford, D.L., D.K. Law and J.K. Babbit. 1972b. Yield and acceptability of machine separated minced flesh from some marine food fish. J. Food Sci. 37:551.

Cross, C.K. and P. Ziegler. 1965. A comparison of the volatile fractions from cured and uncured meat. J. Food Sci. 30:610.

Dambergs, N. 1963. Extractives of fish muscle. 3. Amounts, sectional distribution and variation in fat, water-solubles protein and moisture in cod fillets. J. Fish Res. Bd. Canada 20:909.

Dawson, L.E., D. Bigbee and N. Sobel. 1963. An edible acetylated monoglyceride coating for poultry meat preservation. Poultry Sci. 42:197.

Dawson, L.E., T. Pierson and P. Tack. 1975. Part Five-Food Technology. In Proc. Underutilized Fresh Water Fish Species. E. Lansing, MI June 25-26.

Deng, J.C., R.F. Matthews and C.M. Watson. 1977. Effect of chemical and physical treatments on rancidity development of frozen mullet (Mugil cephalus) fillets. J. Food Sci. 42:344.

DeWitt, K.W. 1963. Seasonal variation in cod liver oil. J. Sci. Food Agr. 14:92.

Dugan, L.R., H.R. Kraybill, L. Ireland and F.C. Vibrans. 1950. Butylated hydroxyanisole as an antioxidant for fats and foods made with fat. Food Technol. 4:457.

Dyer, W.J. 1974. Stability of minced fish an important factor in standards formulation. In Proc. 2nd Technical Seminar of the Mechanical Recovery and Utilization of Fish Flesh. Boston, MA June 12-13.

Eriksson, C.E., P.A. Olsson and S.G. Svensson. 1971. Denatured hemaproteins as catalysts in lipid oxidation. J. Am. Oil Chem. Soc. 48:442.

Farr, A.J. and K.N. May. 1970. The effect of polyphosphates and sodium chloride on cooking yields and oxidative stability of chicken. Poultry Sci. 49:268.

Fischer, T. and J.C. Deng. 1977. Catalysis of lipid oxidation: A study of mullet (Mugil cephalus) dark flesh and emulsion model system. J. Food Sci. 42:610.

Froning, G.W. 1966. Effects of various additives on the binding properties of chicken meat. Poultry Sci. 45: 185.

Froning, G.W. and F. Johnson. 1973. Improving quality of mechanically deboned fowl meat by centrifugation. J. Food Sci. 38:279.

Gibson, T.A., R.E. Worthington, E.K. Heaton and L.R. Beuchat. 1977. Quality evaluation of frozen stored channel catfish grown by tank culture: Effects of dietary fat, freezing method and storage temperatures. J. Food Sci. 42:352.

Greig, R.A. 1965. Technological investigation of pond research fish. 2. Extension of the shelf life of Buffalofish products through the use of antioxidants. Fish. Ind. Res. 2(4):1.

Greig, R.A., J.A. Emerson and G.W. Fliehman. 1967. Extending the shelf life of frozen Cisco (Coregonus artedii) products through the use of water-soluble antioxidants. Fish Ind. Res. 3(4):1.

Gruger, E.H., R.W. Nelson and M.E. Stansby. 1964. Fatty acid composition of oils from 21 species of marine fish, freshwater fish, and shellfish. J. Am. Oil Chem. Soc. 41:662.

Hansen, P. 1964. Fat oxidation and storage life of iced trout. II. The influence of sex and season. J. Sci. Food Agr. 15:344.

Haymon, C.W., E. Brotsky, W.E. Danner, C.W. Everson and P. A. Hammes. 1976. Frozen cooked meat antioxidant: Improved action of sodium tripolyphosphate with lemon juice concentrate. J. Food Sci. 41:417.

Hiner, R.L., D.M. Gaddis and O.G. Hawkins. 1951. Effect of methods of protection on palatability of freezer-stored meat. Food Technol. 5:223.

Iredale, D.G. 1975. Promoting the utilization of freshwater fish with particular emphasis on species of low commercial value. In Proc. Underutilized Freshwater Fish Species. E. Lansing, MI. June 25-26.

Iredale, D.G. and R.K. York. 1977. Effect of chemical additives on extending the shelf life of frozen minced sucker (Catostomedae) flesh. J. Fish. Res. Bd. Canada 34:420.

Jacobson, M. and H.H. Koehler. 1970. Development of rancidity during short-time storage of cooked poultry meat. J. Agr. Food Chem. 18:1069.

Jakobsson, B. and N. Bengtsson. 1972. A quality comparison of frozen and refrigerated cooked sliced beef. I. Influence of storage and processing variables. J. Food Sci. 37:230.

Jarenback, L. and A. Liljemark. 1975. Ultrastructural changes during frozen storage of cod. I. Structure of myofibrils as revealed by freeze etching preparation. J. Food Tech. 10:229.

Karel, M. 1974. Packaging protection for oxygen-sensitive products. Food Technol. 28(8):50.

Keay, J.N. and R. Hardy. 1974. Application of bone separation techniques to pelagic and underutilized species and the preparation of derived products. In Proc. 2nd Technical Seminar of the Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13.

King, F.J. 1972. Characteristics, uses and limitations of comminuted fish from different species. In Proc. Oak Brook Seminar Mechanical Recovery and Utilization of Fish Flesh. Oak Brook, IL. Sept. 21-22.

King, F.J. 1974. Improving the supply of minced block for the fish stick trade. Marine Fish. Rev. 35(8):1.

Kraybill, H.R., L.R. Dugan, B.W. Deadle, F.C. Vibrans, V. Swartz and H. Rezabek. 1949. Butylated hydroxy-anisole as an antioxidant for animal fats. J. Am. Oil Chem. Soc. 26:449.

Kudo, G., M. Okada and D. Miyauchi. 1973. Gel-forming capability of washed and unwashed flesh of some Pacific coast species of fish. Marine Fish. Rev. 35 (12):10.

Kunsman, J.E. and R.A. Field. 1976. The lipid content of mechanically deboned red meats. J. Food Sci. 41: 1439.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. Crit. Rev. in Food Technol. 2:355.

Lee, C. and R.T. Toledo. 1976. Factors affecting textural characteristics of cooked comminuted fish muscle. J. Food Sci. 41:391.

Lehman, B.T. and B.M. Watts. 1951. Antioxidants in aqueous fat systems. J. Am. Oil Chem. Soc. 28:475.

Maclean, J. and C.H. Castell. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. J. Fish Res. Bd. Canada 21:1345.

Marion, W.W. and R.H. Forsythe. 1962. Protection afforded lipids of turkey meat by butylated hydroxyanisole, egg white solids, gelatin, and tripolyphosphate. Poultry Sci. 41:1663.

Martin, R.E. 1974. Summary of Session V - Research and development update. In Proc. 2nd Technical Seminar on Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13.

May, K.N., R.L. Helmer and R.L. Saffle. 1963. Effect of phosphate treatment on carcass weight changes and organoleptic quality of cut-up chicken. Poultry Sci. 42:24.

Mendelsohn, J.M. 1974. Minced fish is a new form. Marine Fish. Rev. 36(8):34.

Miyauchi, D. 1972. Progress report on minced fish studies at the National Marine Fisheries Service. In Proc. Oak Brook Seminar Mechanical Recovery and Utilization of Fish Flesh. Oak Brook, IL Sept. 21-22.

Miyauchi, D. 1974. Storage studies on minced Rockfish and the use of Carp in minced products. In Proc. 2nd Technical Seminar on the Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13.

Miyauchi, D., M. Patashnik and G. Kudo. 1975. Frozen storage keeping quality of minced Black Rockfish (Sebastes spp.) improved by cold-water washing and use of fish binder. J. Food Sci. 40:592.

Miyauchi, D. and M. Steinberg. 1970. Machine separation of edible flesh from fish. Fish. Ind. Rev. 6:165.

Moerck, K.E. and H.R. Ball. 1974. Lipid autoxidation in mechanically deboned chicken meat. J. Food Sci. 39: 876.

Molidina, K.H., J.M. Regenstein, R.C. Baker and K.H. Steinkraus. 1977. Effects of antioxidants and chelators on the stability of frozen stored mechanically deboned flounder meat from racks after filleting. J. Food Sci. 42:759.

Moore, S.L., D.M. Theno, C.R. Anderson and G.R. Schmidt. 1976. Effect of salt, phosphate and some non-meat proteins on binding strength and cook yield of a beef roll. J. Food Sci. 41:424.

Morse, R.E. 1955. How phosphates can benefit meat. Food Eng. 27(10):84.

Mountney, G.J. and E.C. Arganosa. 1963. The effect of phosphates on moisture absorption, retention, and cooking losses of broiler carcasses. Poultry Sci. 42:384.

Nakayama, T. and M. Yamamoto. 1977. Physical, chemical and sensory evaluations of frozen-stored deboned (minced) fish flesh. J. Food Sci. 42:900.

Okada, M., D. Miyauchi and G. Kudo. 1973. Kamaboko - the giant among Japanese processed fishery products. Marine Fish. Rev. 35(12):1.

Orthoefer, F.T. 1974. Antioxidant and processing treatment to prevent rancidity in mullet. In Proc. 26th Annual Sessions of the Gulf and Caribbean Fisheries Institute. New Orleans, LA. Oct., 1973.

Patashnik, M., D. Miyauchi and G. Kudo. 1973. Smooth white spread from separated fish flesh from a base for flavored dips and snack items. Food Prod. Dev. 7(6): 82.

Patashnik, M., D. Miyauchi and G. Kudo. 1974. Controlling bone particle content in minced fish muscle. Marine Fish. Rev. 36(8):37.

Patashnik, M., D. Miyauchi and G. Kudo. 1976. Objective evaluation of texture of minced Black Rockfish (Sebastes spp.) during frozen storage. J. Food Sci. 41:609.

Ramsey, M.B. and B.M. Watts. 1963. The antioxidant effect of sodium tripolyphosphate and vegetable extracts of cooked meat and fish. Food Technol. 17:1056.

Schultz, H.W. (ed.) 1962. Symposium of Foods: Lipids and Their Oxidation. AVI Publishing Co., Inc., Westport, Ct. p. 442.

Schwartz, W.C. and R.W. Mandigo. 1976. Effect of salt, sodium tripolyphosphate, and storage on restructured pork. J. Food Sci. 41:1266.

Shults, G.W., J.J. Howker and E. Wierbicki. 1976. Effect of salt and sodium tripolyphosphate in texture organic volatiles and sensory characteristics of irradiated and nonirradiated pork. J. Food Sci. 41:1096.

Sinhuber, R.O. and T.C. Yu. 1958. 2-thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol. 12:9.

Stansby, M. 1973. Polyunsaturates and fat in fish flesh. J. Am. Diet. Assoc. 63:625.

Steagall, E.F. 1965. The EDTA titration of calcium and magnesium. J. Assoc. Off. Agr. Chem. 48:723.

Stemmber, M.H. 1974. German Patent 2,220,685. Food Technol. 28(6):99.

Stuckey, B.N. 1956. Which antioxidant for your fat containing foods. Food Eng. 28(7):76.

Sweet, C.W. 1973. Activity of antioxidants in fresh fish. J. Food Sci. 38:1261.

Swift, C.E. and R. Ellis. 1957. Action of phosphate in sausage product. II. Pilot plant studies of the effects of some phosphates on binding and color. Food Technol. 11:450.

Tarladgis, B.G., B.M. Watts, M.T. Younathan and L.R. Dugan. 1960. A distillation method for this quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44.

Tarr, H.L. 1948. Control of rancidity in fish flesh. II. Physical and chemical methods. J. Fish. Res. Bd. Canada 7:237.

Teeny, F.M. and D. Miyauchi. 1972. Preparation and utilization of frozen blocks of minced Black Rockfish muscle. J. Milk Fd. Technol. 37:414.

Tims, M. and B.M. Watts. 1958. Production of cooked meats with phosphate. Food Technol. 12:240.

Turner, E.W., W.D. Paynter, E.J. Montie, M.W. Bessert, G.M. Struck, F.C. Olson. 1954. Use of the 2-thiobarbituric acid reagent to measure rancidity in frozen pork. Food Technol. 8:326.

U.S. Army. 1975. Investigation of methods for introducing antioxidants into foods. Project DAAG1773 C-0214, Natick Research and Development Command. Natick, MA.

Watts, B.M. 1950. Polyphosphates as synergistic antioxidants. J. Am. Oil Chem. Soc. 27:48.

Watts, B.M. 1961. The role of lipid oxidation in lean tissues in flavor deterioration of meat and fish. In Proc. Flavor Chemistry Symposium, Campbell Soup Co., Camden, NY. p. 83.

Webb, N.B. and F.B. Thomes. 1975. Development of seafood patties utilizing mechanically separated fish tissue. North Carolina Agricultural Experiment Station Tech. Bull. No. 235. Univ. of North Carolina, Raleigh, NC.

Webb, N.B., E.R. Hardy, G.G. Giddings and A.J. Howell. 1976. Influence of mechanical separation upon proximate composition, functional properties, and textural characteristics of frozen Atlantic Croacker muscle tissue. J. Food Sci. 41:1277.

Wierbicki, E., V.R. Cahill and F.E. Deatherage. 1957. Effects of added NaCl, KCl, $CaCl_2$, $MgCl_2$ and citric acid on meat shrinkage at 70°C and of added NaCl on drip losses after freezing and thawing. Food Technol. 11:74.

Younathan, M.T. and B.M. Watts. 1959. Relationship of meat pigments to lipid oxidation. Food Res. 24:728.

Yu, T.C. and R.O. Sinnhuber. 1957. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol. 11:104.

Yu, T.C., R.O. Sinnhuber and D.L. Crawford. 1973. Effect of packaging on shelf life of frozen silver salmon steaks. J. Food Sci. 38:1197.

Zipser, M.W. and B.M. Watts. 1961. Oxidative rancidity in cooked mullet. Food Technol. 15:318.