This is to certify that the

dissertation entitled

COMPOSITION AND STABILITY OF MECHANICALLY DEBONED CARP (CYPRINOUS CARPIO) WITH EMPHASIS ON LIPIDS AND TEXTURE DURING FROZEN STORAGE

presented by

Yathirajulu M. Naidu

has been accepted towards fulfillment
of the requirements for

Doctoral degree in Food Science & Human Nutrition

L. E. Dawson
Major professor

Date October 25, 1983

COMPOSITION AND STABILITY OF MECHANICALLY DEBONED CARP (CYPRINOUS CARPIO) WITH EMPHASIS ON LIPIDS AND TEXTURE DURING FROZEN STORAGE

By

Yathirajulu M. Naidu

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1983

ABSTRACT

COMPOSITION AND STABILITY OF MECHANICALLY DEBONED CARP (CYPRINOUS CARPIO) WITH EMPHASIS ON LIPIDS AND TEXTURE DURING FROZEN STORAGE

By

Yathirajulu M. Naidu

Composition and storage stability of mechanically deboned minced carp and hand filleted carp harvested from the Great Lakes were evaluated following freezing and frozen storage. Emphasis was on changes in lipids and their fatty acids. General stability characteristics were evaluated using TBA analyses, changes in various classes of lipids and fatty acids, shear values, water-holding capacity, protein solubility, and sensory evaluation. Suitability of one or more antioxidant formulations for increasing the shelf life of carp during long-term frozen storage was determined. Different packaging and storage environments to decrease lipid oxidations were evaluated. Seasonal variations in proximate composition and detailed lipid composition of carp harvested from Lake Huron were determined.

The yield of edible meat from the mechanical deboning process (40%) was greater than that from hand deboning (28.5%). Mechanically deboned flesh contained a lower percent protein and a higher percent lipid than did hand deboned flesh. This difference was mainly due to the

neutral lipid fraction. Phospholipids accounted for about 14% of the total lipids and were separated into six fractions of which phosphatidylethanolamine and phosphatidylcholine formed nearly 60% of the total phospholipids. Twenty-five different fatty acids from C_{14} to C_{22} with 0 to 6 double bonds were identified, of which C_{14}, C_{16}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:1}$, $C_{20:4}$, $C_{20:5\omega6}$ & $\omega3$, and $C_{22:6}$ were the predominant fatty acids.

BHA + BHT + ascorbic acid (with or without propyl-gallate) and Tenox 2 were identified as excellent antioxidant formulae for frozen storage of mechanically deboned carp, while Freezgard was found suitable for hand filleted carp. Little advantage of using vacuum and/or nitrogen to increase storage stability was apparent. Based on TBA numbers, retention of unsaturated fatty acids, taste panel evaluation for rancidity, water-holding capacity, shear values, and extractability of soluble proteins, it was concluded that the acceptable quality of mechanically deboned carp can be maintained for about 8 months during frozen storage while fillets with skin can be preserved for 8 months in comparison to 6 months for skinless fillets.

The lowest lipid content of 6.45% was found in carp harvested in April and the highest in carp harvested during July (16.9%). An inverse relationship between

percent total protein and total lipid was observed due to seasonal differences.

To my Dear
Parents,
Brothers
and
Sisters

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation and gratitude to his major professor, Dr. Lawrence E. Dawson, for providing guidance, assistantship support and marvelous friendship during the investigation and preparation of this dissertation. Thanks are extended to Mrs. Dawson.

Appreciation is extended to Drs. A.M. Pearson, J.I. Gray, J.F. Price, C.M. Stine and A.M. Booren of the Department of Food Science and Human Nutrition and Drs. L.L. Bieber and H.A. Lillevic of the Department of Biochemistry for serving on the doctoral committee.

Thanks are due to Dr. M.R. Bennink for providing the use of GLC facilities. To Drs. C.A. Arntzen, J. Duesing (Plant Research Laboratory) and K.K. Baker (Center for Electron Optics) for providing partial research assistantship support during graduate study at Michigan State University.

Sincere appreciation and thanks are given to Rose Gartner, Norbottons, my parents, brothers and sisters.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The commercial fishing industry in the Great Lakes has been declining because of the decreased availability of fish species that are in high demand. One option to revitalize this commercial industry is to promote the use of underutilized fish species such as carp and suckers. The fresh water mullet (sucker) has proven to be an economical source of acceptable fish products (Baker et al., 1977; UGLRC Report, 1978; Lindsay, 1975; and Morris and Dawson, 1979). Carp was not as acceptable as mullet due to various problems such as its poor image, muddy flavor, and pesticide accumulation. Recent studies support the potential for new products from mechanically processed carp (Price et al., 1981; Rippen, 1982).

Acceptance of underutilized fish species can be influenced by a better understanding of the problems associated with various flesh characteristics, composition, and their changes during processing and storage for each species. The use of mechanically deboned minced carp flesh in restructured products might improve utilization potential for carp. The intramuscular bone structure of carp is unsuitable for hand or mechanical filleting (Dawson et al., 1978; Noble, 1974). This can be overcome

by the use of mechanical deboning equipment. Successful utilization of mechanically deboned carp in various restructured products might require different lengths of frozen storage period for the mince. During frozen storage, various chemical changes may occur, depending on flesh composition and type of fish species. This may affect flavor, texture, and other sensory qualities of such products.

Babbitt (1972), King (1962), Miyauchi (1972), Lee and Toledo (1977), and Morris and Dawson (1979) observed that a rapid deterioration of mechanically deboned fish flesh occurs during frozen storage. This deterioration may be greater in mechanically deboned products which have been subjected to tissue destruction, exposure of lipids to heme and nonheme iron, incorporation of oxygen or to increased flesh contact with enzymes. For example, minced flesh with kidney tissue may result in poor texture because of the activity of alkaline protease, which can bring about slow proteolysis even in frozen storage. Volatile compounds associated with skin are incorporated into tissue mince during mechanical deboning, and can accelerate lipid oxidation and can add components which may increase the development of "off" odors (Cole and Keay, 1976; Teeny and Miyauchi, 1972; Bremner, 1977; Patashnik et al., 1973). Mechanical deboning results in mixing the high fat content red muscle, more susceptible to lipid degradation with the

white muscle, thus imparting the deteriorative changes to the whole flesh (Mai and Kinsella, 1979). Texture changes also occur during frozen storage. They may be related to changes in pH, free fatty acid release and various reactions between proteins and lipids and resulting degradative products. These textural changes lead to altered functional characteristics of the mechanically deboned fish flesh during frozen storage. They are major concerns in storage of frozen fish mince (Iredale and York, 1977). Increased information regarding the advantages and disadvantages for storing both carp fillets and mechanically deboned minced Carp should be of value in developing or improving market opportunities.

Different antioxidant systems or application levels vary in effectiveness for different species of fish during frozen storage. Teeny and Miyauchi (1972) found that BHA, BHT, and their combinations were effective in minimizing lipid oxidation in stored trout. Propyl gallate has been shown to be very effective for bullfish (Greig, 1967). Acetylated monoglyceride was used as a successful antioxidant for fresh fish (Stemmber, 1975) and Deng et al. (1977) showed that TBHQ was an effective antioxidant for mullet and Morris and Dawson (1979) reported that Freezgard was effective for suckers. Hence, determination of an effective antioxidant system is an initial step for successful frozen storage of carp.

Specific objectives of this study were:

1. to study the yield and compositional characteristics of mechanically deboned carp with emphasis on lipids.
2. to compare the effect of various antioxidants and combination of antioxidants for extending shelf life of carp flesh during frozen storage.
3. to evaluate the changes which take place in lipid composition of carp and to evaluate textural changes during frozen storage.
4. to determine the differences between the quality and stability of mechanically deboned flesh and fillets from carp during frozen storage.
5. to determine the seasonal variation in flesh composition of carp harvested from Lake Huron.

REVIEW OF LITERATURE

During the past decade, there has been an increase in demand for fishery products throughout the world. This trend has accompanied a general decline in the availability of traditionally established high value species of both marine and fresh water fish. In the United States annual per capita consumption of fish increased from approximately 11 pounds in 1960 to 13.7 pounds by 1970 (Miyauchi and Steinberg, 1971). Baker (1978) estimated that as much as 80 percent of the possible utilizable species of fish from the Great Lakes fish were underutilized. Many of these species are unintentionally caught and are discarded either because of unestablished markets or because of unavailable processing and handling procedures. Some species of fish may be underutilized because of the presence of intramuscular bones that pose problems for hand or mechanical filleting (Dawson et al., 1978; Noble, 1974). Odd shapes and sizes of fish have been identified as problems in mechanical handling of some fish in commercial processing (Bremner, 1977). Keay (1979) and Teeny and Miyauchi (1972) pointed out that small-sized species would be uneconomical to process by conventional methods.

In addition to the above-mentioned problems leading to the underutilization of potentially available fish species, appearance, names, flesh color and flavor are other factors resulting in underharvesting or discarding of these species harvested accidentally (Baker, 1978; King, 1978; Teeny and Miyauchi, 1972; Patashnik et al., 1974).

Mechanical Deboning

Since the introduction of mechanical deboners in 1940 in Japan, industrial production of restructured fish products has increased significantly (Lanier and Thomas, 1978). This process involves passing of headed, gutted, split, and washed fish between a pressure belt and a revolving perforated drum resulting in separation of flesh that is squeezed through small perforations from bone, skin and scales which do not pass through perforations. Lanier and Thomas (1978) described in detail the design and operation of a fish deboner, and their use can pave the way for commercial utilization of such fish with intramuscular bones that are not suitable for hand or mechanical filleting. Within reasonable limits, most fish, irrespective of their sizes and shapes, can be effectively deboned. The effectiveness of deboning is very noticeable in case of predominantly boney species of fish. Bone contents of 0.13 to 0.16 percent in mechanically

deboned fish flesh were reported by Zapata (1978) and Apolinario (1975).

The greatest advantage of mechanical deboning of underutilized fish species is that it allows the incorporation of the mince into various restructured products in various proportions. This leads to increases in the possibility for the development of new products with specified texture and flavor, and reduces the chances for undesirable products based on factors like species identity, flavor, texture, appearance, and color (Bligh and Regier, 1976; Hewson and Kemmish, 1976; Jaurequi, 1978; Moledina et al., 1977).

One major advantage attributed to the use of mechanical deboner is the increased yield of the edible meat. Flesh yield depends on the type of fish being deboned. Yields from 32 to 70 percent have been reported for various fish species. Reseck and Waters (1979), Kudo et al. (1973), and Noble (1974) reported a 70 percent yield for a small boney fish, whereas a yield of 25 to 30 percent was found for hand deboned products (Miyauchi and Steinberg, 1971). The left over fish frames from mechanical filleting can be mechanically deboned to recover edible meat using mechanical deboning (King and Carver, 1971; Moledina et al., 1977; Noble, 1973).

Miyauchi et al. (1975) and Bremner (1977) have shown that variations in the mechanical deboner, such as belt

pressure and size of perforations of the drum, can influence the yield of edible flesh, bone, skin, and scale content.

The use of mechanically separated fish flesh in restructured fish products requires more attention to quality changes which occur during processing and storage. Lee and Toledo (1977), Babbitt (1972), King (1972) and Miyauchi (1972) indicated that fish flesh deteriorates rapidly after mechanical deboning, with major changes occurring in flavor and color. Some of the theories proposed for these accelerated adverse changes in mechanically deboned fish muscle include incorporation of soluble constituents from skin and bone marrow and mixing of oxidation susceptible lipid fractions from flank muscles (Watts, 1954; Blackwood, 1974; Steinberg, 1975). Direct contact between fish flesh and iron parts of a mechanical deboner was reported to induce accelerated lipid oxidation (Castell et al., 1966; Lee and Toledo, 1977).

The aging of some fish from 1 to 4 days in ice can lead to an increase or decrease in acceptable fish flavor. Raja and Moorjani (1971) attributed such flavor improvement to the formation of inosine 5 monophosphate (IMP) by enzymatic deamination and phosphorylation of ADP.

Mechanical deboning can directly influence flavor change in fish products. Such changes were associated with the inclusion of soluble and volatile compounds extruded from skin during mechanical separation (Crawford

et al., 1972) and inclusion of organel tissue and peritoneal membrane which have been reported as a basis for off flavor in mechanically deboned meat (Dingle and Hines, 1975). Blood may impart a metallic flavor to minced fish product (Keay, 1979). A rupture of tissues and release of cell contents can accelerate enzyme induced flavor changes (Howgate, 1976).

A blend of spices and smoke flavor ingredients can improve the flavor of mechanically deboned carp (Patashnik et al., 1974), while a mix of other acceptable fish mince or minced shrimp has been recommended for improving flavor (Babbitt et al., 1974). Mahohar et al. (1973) reported that polyphosphates may not effectively and consistently improve flavor.

Composition of Fish

The proximate composition of fish muscle varies widely due to species (Stansby and Olcott, 1963), nutrient availability (Cutting, 1969), season (Finne et al., 1980), or location (Deng et al., 1976). Composition may vary from 30 to 90 percent moisture, 6 to 28 percent protein, 0.1 to 65 percent fat, and 0.3 to 1.7 percent ash.

Fish have been classified based on protein and fat content into five categories (Stansby and Olcott, 1963). These categories are (1) low fat, high protein fish; (2) medium fat, high protein fish; (3) high fat, low protein

fish; (4) low fat, low protein fish; and (5) low fat, very high protein fish. Respective examples are cod, salmon, trout, clam, and tuna.

Seasonal Variation

Generally, migratory fish have highly significant differences in proximate composition among seasons. This also applies to most other fish with high fat content. Changes during the year in availability of food, temperature of water, diet or physical, chemical, and physiological changes brought about by reproductive cycle, play a significant role in proximate composition of the species (Sidwell, 1976; Stansby and Lemon, 1941). In most species, principle constituents affected are moisture and lipid content followed by protein (Leu et al., 1981). With these variations, components of each constituent can undergo profound changes. For example, individual fatty acids or fractions of phospholipids of the total lipid, can vary greatly. Contents of moisture and total lipids shows an inverse linear relationship to each other (Venkataraman et al., 1968; Leu et al., 1981). Generally, it has been observed that monoenes are least affected by seasonal variation while the polyenes are affected the most. Venkataraman et al. (1968), Deng et al. (1976) and Leu et al. (1981) observed that the high lipid content coincides

with the prespawning period while low lipid content is found after spawning.

Fat in Fish Flesh

Stansby (1973) reported that "Next only to moisture, the fat content of fish varies more than any other chemical components. Although the range in fat content can vary, the content of individual species may range from 0.5 to 25 percent. A majority of fat in fish occurs in the form of phospholipids and triglycerides. More of the fatty acids in phospholipids are generally polyunsaturated than those in triglycerides. In some fish species, most of the fat appears as phospholipids comprising basic cellular components. However, in fish with high fat content, the fat exists as fat depots at various locations throughout the body". In some fish, significant amounts of fatty acids may occur in unusual forms. For example, 20 percent of fatty acids occur as alkoxydiglycerides in sharks and there are known cases where they occur as wax esters (Stansby, 1973).

Fat content may vary from very low percentages in edible flesh to high percentages in liver and ovaries. Most fish have, in addition to intracellular phospholipids, a store of triglycerides which are found in layers beneath the lateral line in skin along the dorsal and ventral areas (Liciardello *et al.*, 1980).

During any one season of the year, individuals of a species, even from the same catch, may vary considerably from other fish in fat content. Maximum triglyceride content has been reported during summer months and minimum content has been observed just before the end of winter (Deng et al., 1976).

The fat content of muscle from different parts of a fish differs considerably; for example, a steak taken from the tail end of the fish may contain 10 to 60 percent less fat compared to a similar steak taken from the area adjacent to the head, depending on species of fish. Belly flaps usually contain higher fat contents than remainder of fish (Leu et al., 1981).

The fat content of red or dark muscle of fish is higher than that of white or light muscle and the fat from red muscles is known to be more susceptible to oxidative rancidity (Lee and Toledo, 1977).

In general, more than 20 fatty acids are present in a species of fish, varying in chain length from C_{14} to C_{24} and occasionally include C_{12} and C_{26}. Twenty to thirty percent of the fatty acids are saturated and a majority are C_{16} and C_{18} fatty acids. From thirty to sixty percent of the fatty acids are monoenes. Polyenes of C_{16}, C_{18}, C_{20}, C_{22} form the major portion of polyunsaturated fatty acids. C22:5, C22:6, C20:5 and C20:4 form major portions of polyunsaturated fatty acids in most fresh water fish

species (Stansby, 1973).

Kinsella et al. (1977) determined the fatty acid content of 18 species of fresh water fish. They reported a marked variation in fatty acid composition among various species. They also reported that at any given time, large fish contained a higher fat content than small fish within the sampled species and catch.

Hayashi and Takagi (1977) reported that stress can influence the fatty acid content of fish. They reported that gill netted fish had a lower phospholipid to neutral lipid ratio than did trawl netted fish, with significant decreases in C20:5 ω 3 and C22:6 ω 3 acids. This was attributed to special physiological conditions caused by excessive stress.

Protein of Fish Muscle and Texture

The protein content of fish may vary from 6 to 28 percent, depending on the species, season, and the stage of reproductive cycle (Stansby and Olcott, 1963). A major percentage of protein nitrogen is myofibrillar protein (58 to 73 percent), followed by the soluble sarcoplasmic protein (20 to 30 percent) and stromal protein (3 to 10 percent) (Moorjani et al., 1962). Myofibrillar proteins contain 40 to 60 percent myosin and 20 to 40 percent tropomyosin (Dyer and Dingle, 1961).

Preservation of fish by freezing causes protein denaturation leading to a marked effect on the textural quality of the meat. These changes occur rather slowly in frozen fish but significantly affect the acceptability of texture (Hao Chu and Sterling, 1970). Depending on the condition of the flesh and rate of freezing, ice crystals of various sizes can form inside and outside the cells resulting in mechanical damage to muscle cells (Love, 1968; Kent, 1975). Increased salt concentrations due to a decrease in available water could result in protein denaturation (Castell et al., 1970; Sikorski et al., 1976). Ca^{++} and Cu^{++} ions have been shown to bring about protein insolubilization by favoring protein lipid interactions (Pigott and Shenouda, 1975; Buttkus, 1971).

Lipid hydrolysis can cause protein denaturation. Lipid oxidation, resulting in the formation of free radicals may also lead to protein deterioration. Dyer and Dingle (1961) reported a decrease in fish myofibrillar protein extraction with increased free fatty acid formation. The free fatty acids formed may bind polypeptide side chains and polar groups in intermolecular hydrophillic and hydrophobic ionic linkages and decrease the protein solubility.

Polymerization of protein may lead to decreased solubility during lipid oxidation (Funes and Karel, 1981; Tappel, 1961). Availability of water may control the extent of polymerization (Schaich and Karel, 1975), thus

the method of freezing is important.

Denatured proteins exhibit poor waterholding capacity which leads to lower product yields and brings about deteriorative effects on texture, flavor, color, and nutritional characteristics of fish products (Eskin et al., 1977).

Lipid Oxidation and Stability

The process of spontaneous oxidation on exposure to the air is by no means limited to lipids from foods. It is exhibited by various chemicals, such as hydrocarbons, aldehyde ethers, sulfhydryl compounds, phenols, amines, and sulfites (Labuza, 1971). Among the constituents of foods susceptible to autoxidation are the unsaturated fatty acids in their various forms or combinations and minor constituents which influence aroma, flavor, color and vitamins (Lea, 1962).

Factors Affecting Lipid Oxidation

One characteristic feature of lipid autoxidation is that it can be influenced by factors other than the usual variations in concentration of reactants and temperature. Some of the most important factors affecting lipid oxidation are listed below.

Lipid Composition

Lipid composition involves the nature and proportions of the unsaturated fatty acids present (Lea, 1962). Since double bonds are the active sites of oxidation, fatty acids vary in their reactivity depending on their degree of unsaturation (Privett, 1959). More specifically, autoxidation of a particular lipid will depend on its content of unsaturated fatty acids and their degree of unsaturation. Since the fatty acid composition of fish varies with season, diet, and the stage of reproductive cycle the fish is undergoing, these factors can indirectly play a marked role in autoxidation.

Light

Ultraviolet and blue lights impart a significant influence on lipid autoxidation (Lea, 1962; Sherwin, 1968). Light falling in these wave length can accelerate the rate of lipid autoxidation. One of the primary effects seems to be the acceleration of hydroperoxide decomposition in fats (Lundberg, 1962).

Ionizing Radiation

Alpha and beta particles and X-rays have definite effects on the autoxidation rate of lipids (Chipault, 1962). They can catalyze peroxide decomposition and formation of free radicals (Lundberg, 1962).

Peroxide

The peroxides present in the food system and those formed as a result of initial autoxidation of lipids play an important role in the further initiation and fate of autoxidation reactions (Lea, 1962; Sherwin, 1968).

Enzymes

Lipoxygenases (lipoxidases) influence the rate and course of fat oxidation (Lea, 1962; Tappel, 1962). Enzymes are also known to be involved in initiation of fat autoxidation (Clegg et al., 1953).

Organic Iron and Trace Metal Catalysts

This classification includes hematin compounds such as hemoglobin and heavy metals such as Co, Cu, Fe, Mn, and Ni. Detailed reviews involving such catalysts were reported by Ingold (1962), Lundberg (1962), and Tappel (1962).

In addition to the factors mentioned above, consideration of the method of catch, physical state prior to processing, handling conditions and storing temperatures might influence physical/chemical changes in fish muscle.

Mechanism of Lipid Oxidation

Proposed mechanisms and theories of lipid oxidation have been reviewed by Privett (1959), Tarladgis (1961), Lundberg (1962), Keeney (1962), Ingold (1962), Labuza (1971), and Sherwin (1972).

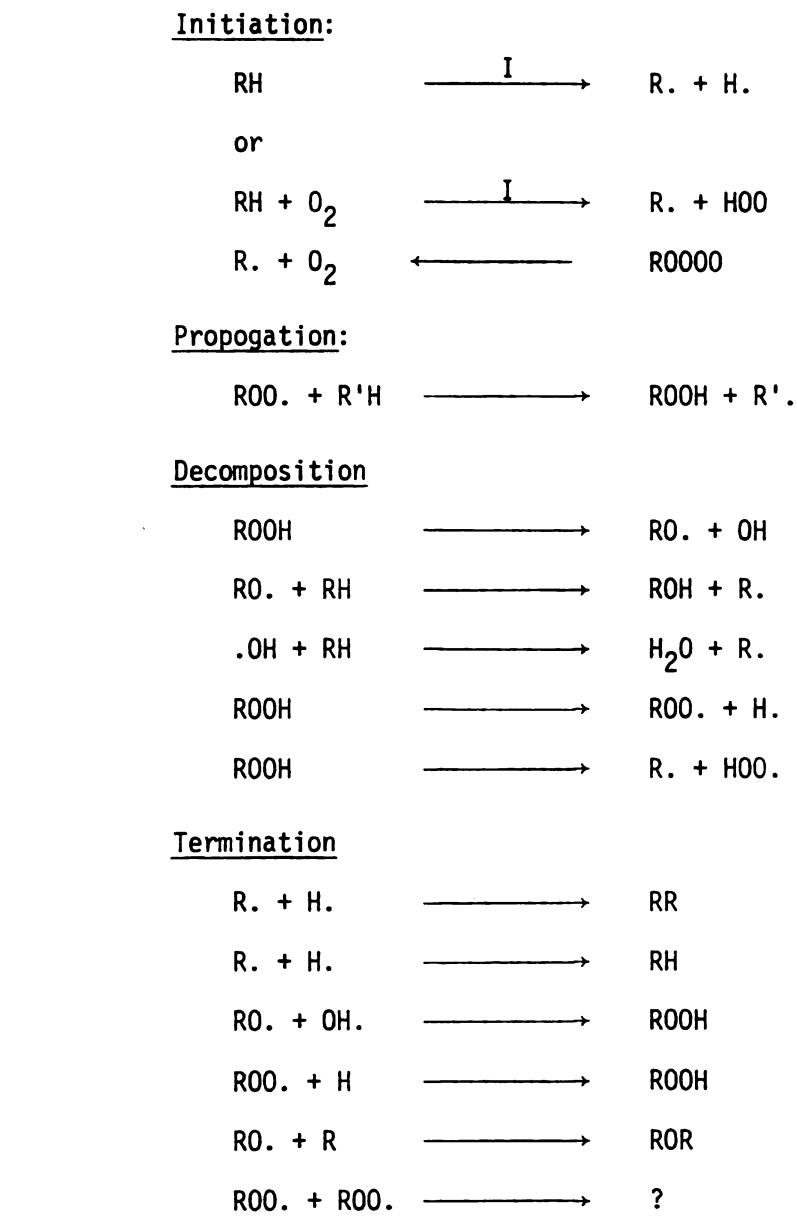
According to Lundberg (1962) the oxidation of food lipids involves autoxidative reactions which are accompanied by various secondary reactions having oxidative or nonoxidative character. Autoxidation is defined by the same author as the reaction of any material with molecular oxygen.

The principal mechanisms involved in the primary autoxidation of fatty materials have been elucidated fairly well. Lundberg (1962) stated that, "A large number of secondary reactions, many of which are virtually unstudied are also involved in the oxidation deterioration of lipid materials". He also reviewed and discussed the general features and kinetics of oxidation of fats. "First, it is an autocatalytic reaction, i.e. a reaction whose rate increases with time due to formation of products which themselves catalyze the reaction. Second, the reaction primarily involves unsaturated acyl groups, and hydroperoxide groups appearing in alpha position relative to a double bond. Depending on the amount of original unsaturation in the acyl group and other factors, the formation of hydroperoxides may or may not involve the shift of a double bond. Third, among the common fatty acids and their derivatives, the rates of autoxidation are greatly dependent on the degree of unsaturation of the fatty acids. Fourth, various extraneous influences may be present that affect the rate of oxidation. Some of these factors have been

presented earlier. Fifth, as with other chemical reactions, temperature has a marked effect on the rate of autoxidation. Sixth, although the major products of fat oxidation are hydroperoxides, certain secondary products not derived from peroxides are formed concurrently. Finally, hydroperoxides themselves do not contribute directly to any appreciable extent to the undesirable flavors and odors of autoxidized foods. Rather, the rancid flavor and odors are due to a host of secondary substances derived through various reactions and further oxidation of peroxides and their degradation products".

Following his kinetic studies on lipid oxidation, Lundberg (1962) presented a free radical chain mechanism for lipid autoxidation. A generalized form of this almost universally accepted theory of autoxidation is presented in Figure 1. Privett (1959) presented an essentially similar mechanism. Figure 1 shows that an initiator (light, heat, heavy metal, enzymes) catalyzes the removal of hydrogen atom from an unsaturated fatty acid (RH), producing a free radical (R.). A molecule of oxygen combines with the free radical to form a peroxy radical (ROO.) which can remove a hydrogen from a new fatty acid (R'H) and thus result in propagation of this chain reaction.

The effect of increasing temperature on the rate of catalytic autoxidation is somewhat greater than in most chemical reactions. Increasing temperature accelerates



Note: I = Heat, metals, light, enzyme
R. = Alkyl free radical
RR = Polymer
ROO. = Peroxy radical
ROOH = Hydroperoxide

Figure 1.--Mechanism of Auto Catalytic Autoxidation.

not only the chain propogation reactions, but also the peroxide decomposition, thereby making a greater concentration of free radicals available for the initiation and further propogation of this reaction chain (Lundberg, 1962). Similar reactions were also illustrated by Sato and Herring (1973) and Dugan (1976).

Various mechanisms have been proposed for the effects of trace metals on oxidation (Lundberg, 1962; Ingold, 1962). The following mechanisms were reported by Lundberg (1962), first a "reduction activation", involving the oxidation of a metal ion (M^{n+}), as well as the production of hydroxyl ions and free radicals. A second mechanism involves reaction of metal ions, which may initiate reaction chains. A third mechanism involves reaction of molecular oxygen with metals to form complexes, which give rise to subsequent formation of hydroperoxide (HOO.) radicals. Yet another mechanism that has been found applicable in some cases is the formation of free radicals by direct reaction of metal ions with an olefinic substrate. A schematic view of some of these mechanisms is shown in Figure 2. Other effects of trace metals on hydroperoxide decomposition and on termination reactions have also been discussed by Ingold (1962). Mechanisms involved in the peroxidation of fatty materials by biological catalysts have been investigated and reviewed by Tappel (1961, 1962). In the latter report schemes are presented for better understanding of such mechanisms.

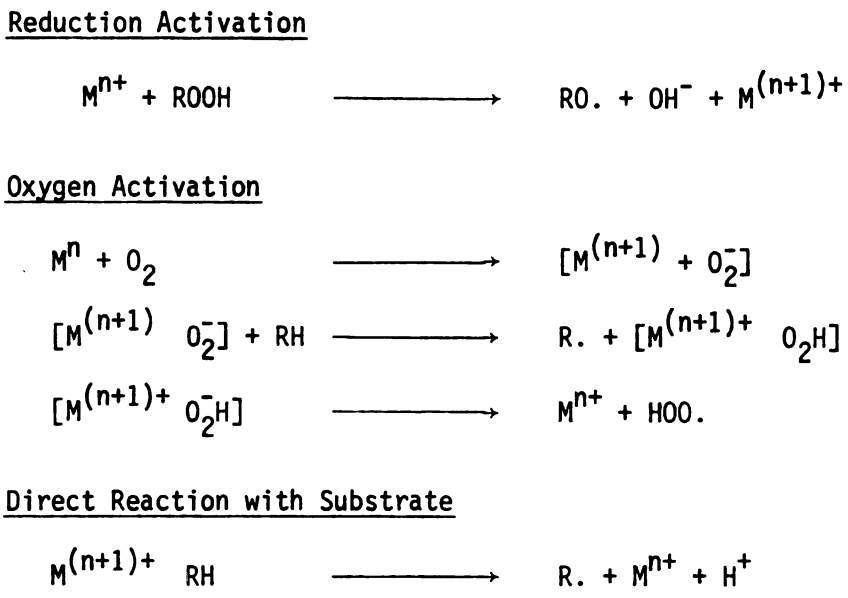


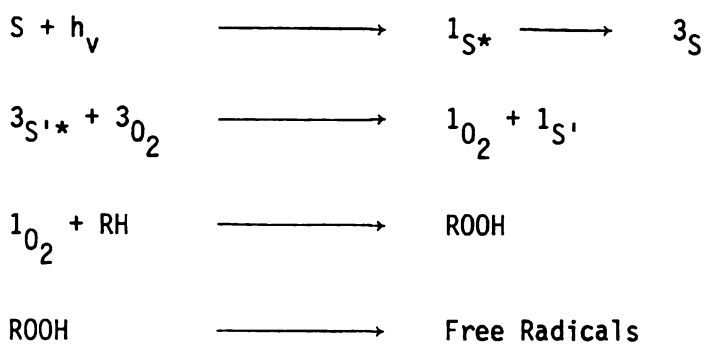
Figure 2.--Mechanism for Metal Catalysis Reaction in Lipid Oxidation.

Frankel (1962) presented a rather extensive review on isolation methods for hydroperoxides, and on their purity determination, characterization, and decomposition. Keeney (1962) reviewed and presented illustrations of the secondary products from degradation of hydroperoxides. Several of the major possible pathways were considered. As previously indicated, lipid autoxidation is an extremely complex process. This complexity results from various simultaneous reactions occurring and their interacting reactants and products (Privett, 1959).

Role of Singlet Oxygen in Lipid Oxidation

Singlet oxygen has been proposed as an intermediate reaction in photo-sensitized initiation which differs from free radical initiated autoxidation reactions. The presence of hydroperoxides with nonconjugated double bonds in the primary reaction indicates participation of singlet oxygen in photosensitized initiation of autoxidation (Vianni, 1980). β-carotene (a singlet oxygen quencher) can inhibit photosensitized initiation of autoxidation, but butyl hydroxytoluene (BHT) (a free radical inhibitor) cannot inhibit this reaction showing that photosensitized oxidation is a different process (Van Santen, 1970).

A general mechanism of singlet oxygen in the process of photosensitized initiation of lipid oxidation as proposed by Rawls and Van Santen (1970) is represented in Figure 3.



* Excited form
1 Singlet state
3 Triplet state
S Sensitizer

Figure 3.--Mechanism of Singlet Oxygen Formation.

Singlet oxygen reactions could be 1.5×K times faster than the triplet oxygen initiation, hence could be an important factor in lipid oxidation.

Hydroperoxide formation has been mainly influenced by singlet oxygen in milk lipid oxidation in the presence of light, copper and xanthine oxidase (Aurand et al., 1977). In light induced oxidation reactions, riboflavin acts as a sensitizer through the production of singlet oxygen (directly from its photosensitized triplet state). Singlet oxygen formation occurs by dismutation of the super oxide anion (O_2^-). In this system oxidation was prevented by using singlet oxygen quenchers showing the participation of single oxygen initiation. Cort (1974) reported that in the absence of photosensitizers, singlet oxygen does not significantly influence lipid oxidation initiation. It is important to note that free radical terminators such as BHA or BHT are of little use in stopping initiation of photosensitized lipid oxidation.

Evaluation of Lipid Autoxidation and Stability

Numerous objective methods have been developed for the evaluation of lipid autoxidation and stability (Erickson and Bowers, 1974). However, in any particular evaluation using an objective method, correlation with a sensory evaluation of the food product is essential. It is important to recognize that when a direct method is used

for detection of lipid deterioration in a food product, the food should be prepared, packaged and stored under the normal conditions that are planned for its future storage. The product should be examined periodically by use of proven sensory evaluation methods (Erickson and Bowers, 1974). Sensory or taste panel evaluations are subjective methods of evaluation and rely on subjective judgment of individuals. Nevertheless, proper training of personnel and use of appropriate statistical sampling and evaluation procedures can result in an objectivity approaching that which is expected from chemical analyses (Erickson and Bowers, 1974). Use of a sensory panel may well be the only reliable option available.

Erickson and Bowers (1974) classified the existing objective methods for the evaluation of lipid autoxidation and stability into four groups. In the first group are those methods based on lipid composition, that is, the fatty acid composition, their relative proportions, and unsaturation. The second group include those methods based on the absorption of oxygen, an indicator of progress of lipid oxidation. The third group comprises those methods based on the intermediate compounds formed, for example, peroxides as an index of progress of oxidation. The fourth group includes methods involving the measurement of one or more of the final reaction products resulting from the peroxide decomposition (Figure 4). These authors

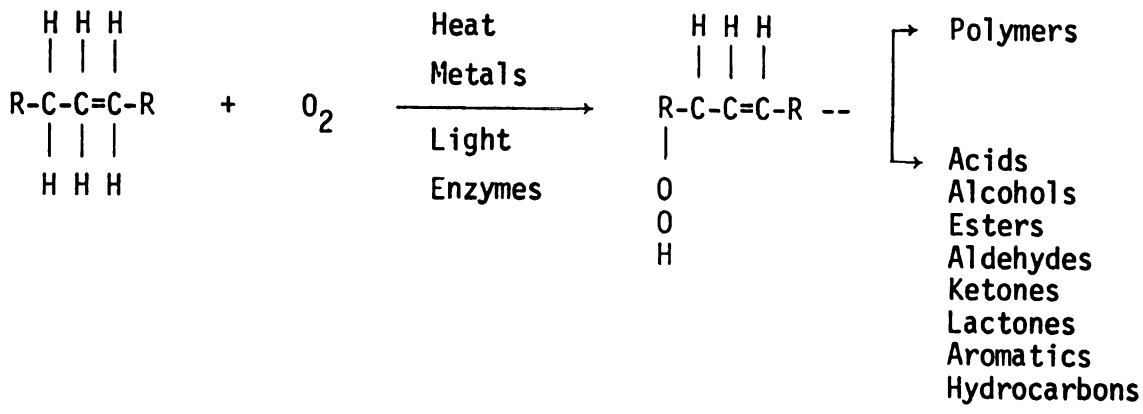


Figure 4.--Some Final Products of Peroxide Degradation.

pointed out that the oxidation process in a lipid system is a dynamic or continuous series of reactions; therefore, any individual determination is subjected to the relative dynamic parameters involved.

Percent Lipid Composition

These methods include the determination of the native proportion and unsaturation degree of the unsaturated fatty acids present in the food. Generally speaking, the higher the proportion and degree of unsaturation of the fatty acids, the more liable the lipid system is to oxidation (Dugan, 1955). Usually the techniques used to evaluate lipid oxidation involve thin-layer and gas chromatography. Such procedures are very attractive for pure lipid systems. However, problems may arise from the isolation of lipids from complex systems. A reduction in the surface for attack by oxygen, artifacts introduced by impure solvents, failure to exclude oxygen and incomplete extraction of fraction components involved, preclude or increase the difficulty of using such methods for processed foods.

Oxygen Absorption

Since oxygen is a reactant in lipid oxidation, the measurement of its uptake may constitute an objective method for lipid oxidation assessment. Nevertheless, due to very low threshold values of many off flavor compounds which result from lipid oxidation, it is apparent that

such compounds could be formed without any measureable oxygen uptake, a fact that limits accelerated oxygen absorption methods. Examples of such methods are the active oxygen method--A.O.M. (A.O.C.S., 1974), an oxygen-absorption method proposed by Eckey (1946), the oxygen bomb method (Blackenship et al., 1973) and monometric oxygen absorption (Johnston and Frey, 1941; Lancaster et al., 1956). In each of these procedures, and for any product, it is necessary to develop an absorption curve and correlate its value with an appropriate sensory evaluation. One final caution, which must be considered for complex systems such as prepared foods, is the fact that the nonlipid constituents may also absorb oxygen (Erickson and Bowers, 1974).

Peroxide Formation

Peroxide formation methods are of very limited use in evaluating oxidation in prepared foods although suitable for pure fat systems. Peroxide formation is a direct result of oxygen absorption. However, peroxides are intermediates in lipid autoxidation. At any time, the amount of peroxides present is a function of the rate of their formation and decomposition. If these rates are the same, significant development of off-flavors occur in a relatively short time. Alternately, if the rate of decomposition is retarded, peroxides may accumulate without affecting flavor since they are essentially flavorless.

An example of a method in this group is the peroxide value determination (A.O.C.S., 1974), which is also used in the active oxygen method.

Decomposition of Peroxides

These methods involve the measurement of products formed during the decomposition of peroxides. Unfortunately, there is an array of such products (Figure 4) and some of them can cause off-flavors at very low concentrations. Extensive time and money involved for accurate determinations of such low concentrations may limit their use as an index of lipid oxidation (Forss, 1973). Examples of methods in this group are the carbonyl test (Henick et al., 1954; Dugan, 1955; Chang and Kummerow, 1955; McKerrigan, 1957; Lea and Swoboda, 1958), and the 2-thiobarbituric acid (2-TBA) test (Tarladgis et al., 1960, 1962, 1964; Tarladgis and Watts, 1960; Pohle et al., 1964; Jacobson et al., 1964; Yu and Sinnhuber, 1964, 1967; Ho and Brown, 1966; and Marcuse and Johansson, 1973). Other methods, using ultraviolet and infrared spectrophotometry, polarography and gas chromatography have been proposed for measuring products from peroxide decomposition (Dugan, 1955; Scholz and Ptak, 1966; Sherwin, 1968; Jarvi et al., 1971). This application, however, is rather limited unless only one fractional component is evaluated and this requires a parallel sensory measurement for assessment of correlations.

Predictive Methods for Evaluation of Lipid Oxidation and Stability

Several choices are available to an investigator in selecting a method for predicting lipid stability. However, most procedures are not as sensitive in the early stages of off-flavor development as is sensory evaluation. Certain foods require extended periods of time for the development of off-flavors. Thus a practical test used under accelerated conditions of off-flavor development and its coupling with a chemical method are essential (Erickson and Bowers, 1974). This acceleration is achieved by introducing one or more factors of autoxidation such as oxygen, temperature and photosensitization. It is important to avoid completely an artificial situation such as ultraviolet light as a factor of acceleration. Another important consideration is that, in accelerated situations, the actual kinetics and some of the reaction pathways are probably different (Moser et al., 1965; Erickson and Bowers, 1974). When elevated temperatures are involved in addition to unsaturated fatty acids, the saturated fatty acids may actively take part in reactions with oxygen (Erickson and Bowers, 1974). This method of prediction is useful only when evaluating pure oils, fats or fried foods with low moisture and which are stored at room temperatures or refrigerated temperatures. This method is not applicable

for stored frozen foods (Erickson and Bowers, 1974).

Evaluation of Methods

Due to the variety of methods available for determining rancidity and stability of fats and fatty foods, the choice of a method for a particular food may be difficult. This choice may be dictated by equipment availability, nature of the problem, financial consideration, time, significance, and confidence level desired (Erickson and Bowers, 1974). It is important to note that in spite of all the methods available and the development of new ones, no single ideal method applicable to all products and problems exist.

Sensory Evaluation of Lipid Oxidation

Taste and smell are the only two human senses which have not been relegated to a second position in modern processing and development operations. The development of automated mechanical control, and sophisticated sensing equipment has not only displaced the other human senses, but has brought about new standards of quality and uniformity (Evans, 1955).

Sensory panels may fall in three different classes: quality control, grading, and consumer preference panels. Reports are available dealing with each of these and with statistical methods associated with them (Amerine et al., 1965; A.S.T.M., 1968a, 1968b, 1968c). Evans (1955)

reviewed and discussed the various methods of sensory evaluation of fats and oils. He presented an extensive discussion of and recommendations for sample preparation and presentation, methodology testing, panel member selection, sample size, score sheets, interpretation of results, motivation of panelists, taster performance, and supplementary chemical tests. Fioriti et al. (1974) also discussed and compared various objective and sensory methods of measuring fat oxidation.

Effects of Lipid Oxidation on Nutritive Values

From various existing reports and reviews (Kaunitz, 1962; Kummerow, 1962; Klinger, 1974), there is some evidence of toxic effects from consumption of autoxidized and thermally abused fats and oils. Chang and Watts (1952) reported a decrease in unsaturated fatty acids, primarily, oleic acid, when fresh and cured pork, beef, lamb, and turkey were cooked. The losses were not considered important when ordinary cooking was done. Similar findings were reported by Phillips and Vail (1967).

Labuza et al. (1971) reported co-oxidation of some vitamins as an undesirable effect of lipid oxidation, and suggested that cross-linking oxidized lipids with proteins reduced their biological availability. Georgieff (1971) discussed the possibility that an excess of unstable free

radicals without sufficient inhibitors, such as vitamin E, may be linked to some type of cancer. Kummerow (1962) reviewed and discussed the possibilities of carcinogenic activity of heated fats.

Johnson et al. (1957) and Nolen (1972) reported that thermally oxidized oil is absorbed at a lower rate than fresh oils. Rats fed a diet containing 10 to 15 percent thermally oxidized oils had a lower growth rate (Poling et al., 1962; Witting et al., 1957; Johnson et al., 1957; Bottino, 1962). Increased ratio of liver weight to body weight was reported as a consequence of consumption of thermally abused oils by rats (Johnson et al., 1957; Poling et al., 1962). Other results of consumption of thermally abused oils and fats by rats involve reduced fecal nitrogen (Nolen, 1972), loss of hair and discoloration (Bottino, 1962), and in some cases, death (Bottino, 1962; Witting et al., 1957). Matsuo (1962) presented an extensive review of the work concerning the feeding of rats with autoxidized and thermally polymerized fish oils and agreed with the above authors. Poor growth was observed as a result of feeding thermally abused corn oil to chicken (Kummerow, 1962).

Pyridoxine and riboflavin were found to protect rats against poor growth induced by consumption of commercially heat abused fats (Witting et al., 1957). In an eleven-month experiment, rats fed hydrogenated soybean oil which

had been used for 56 hours of frying, showed no adverse effects including offsprings. When lipids are heated excessively, some toxic effects have been reported from feeding studies (Deul et al., 1951; Poling et al., 1962; Chang and Watts, 1962). Poling et al. (1962) warned that there is a potential toxicity when fats are abused excessively. Matsuo (1962) reported negative growth effect on rats from consumption of unheated autoxidized fish oils. This author stressed the point that highly unsaturated fats and oils, even when not heated, may be dangerous due to autoxidation alone.

Antioxidation and Antioxidants

Mechanism of Antioxidation

Antioxidants are substances which react with free radicals formed during lipid autoxidation to give stable products and extend the shelf life of the substance. Antioxidants, however, do not avoid or block autoxidation completely or permanently; they merely retard the chain reaction process; and eventually will be lost from the lipid (Stukey, 1962) or become oxidized (Weiss, 1970).

Lundberg (1962a) reviewed the early work on antioxidation and presented schematically one of the most accepted mechanisms of antioxidation (Figure 5). In any of these mechanisms, antioxidants are proposed to act as hydrogen donors or free radical acceptors. This form of

1. $ROO. + AH_2 \longrightarrow ROOH + AH.$

   $AH. + AH. \longrightarrow A + AH_2$

2. $ROO. + AH_2 \longrightarrow (ROOAH_2).$

   $(ROOAH_2). + ROO. \longrightarrow$ STABLE PRODUCTS

3. $AH_2 + ROO. \longrightarrow AH. + ROOH$

   $AH. + ROO. \longrightarrow ROOH + A$

Figure 5.--Proposed Mechanism of Antioxidation.

action is called "primary" inhibition and an "auxiliary" inhibition involves removal or destruction of hydroperoxides without the formation of chain-initiating free radicals.

Stuckey (1962) referred to four possible mechanisms by which an inhibitor may function as a chain stopper for the free radical mechanisms of lipid oxidation. These are: hydrogen donation of antioxidant, electron donation by the antioxidant, addition of the lipid to the aromatic ring of the antioxidant and formation of a complex between the lipid and the aromatic ring of antioxidant. He presented evidence from the literature which strongly corroborates the first mechanism. However, evidence which favors the other three mechanisms was also discussed.

Although antioxidants have been used successfully to retard lipid oxidation, the exact mechanisms of their action have not been completely clarified.

Metal Sequestering Agents

Metal sequestering agents are called metal scavengers or metal chelating agents. They bind metals and thereby inactivate the participation of trace metals such as copper and iron which are powerful pro-oxidants of fats and oils (Lundberg, 1962b; Weiss, 1970). The most commonly used metal sequestrant is citric acid. Others are isopropyl citrate, stearyl citrate, ascorbic acid, monoglyceride citrate, and polyphosphates.

Synergism and Antioxidants

The antioxidant effectiveness of a mixture of two substances is frequently greater than the sum of the inhibitory effects that are obtained when the same quantity of each antioxidant is used alone. This phenomenon of both substances helping one another is termed synergism (Lundberg, 1962b). In some cases, one of the two substances is far more effective than the other when used alone, and in such cases the more effective material is referred to as primary antioxidant, and the less effective material is referred to as the synergist. However, synergism is frequently observed with substances that have approximately the same order of effectiveness when used alone, and are similar in chemical structure, such as butylated hydroxyanisole and butylated hydroxytoluene.

Various explanations and mechanisms have been proposed for synergism among antioxidants. The acid synergists are also metal chelating agents and the action has been considered as the only explanation for such synergism (Lundberg, 1962a). (Mixed free radical acceptor synergism, however, should receive another explanation.) Other existing theories of antioxidant-synergism were discussed by Lundberg (1962b) and Stuckey (1962).

Antioxidants

According to Weiss (1970) antioxidants were developed primarily for use with pure fats. Presently their use is permitted in many countries for food use and for animal feeds (Lea, 1962). Stuckey (1962) affirmed that the development of antioxidants actually started during World War I with nonfood materials such as rubber, gasoline, and plastics.

Sherwin (1972) stressed the point that antioxidant free radicals, formed when interferring in the autoxidation process, cannot initiate or propogate the oxidation reactions. This same author stated that antioxidants do not function by competing with the substrate for oxygen and that they are not oxygen absorbers or adsorbers, but merely are free radical inhibitors. This seems to be the case, although Weiss (1970) referred to antioxidants as using up available oxygen in the shortenings to which they were added.

Antioxidants, as other additives for use in human foods, are subjected to regulations under the Food, Drug, and Cosmetic Act; the Meat Inspection Act; the Poultry Inspection Act; and the U.S. Food and Drug Administration Food Additive Amendment. The antioxidants permitted are not likely to harm the consumer. The level of antioxidant usage in foods is also regulated (U.S.D.A., 1960).

Chemical Classification of Antioxidants

Antioxidants, other than those having metal-sequestering action, were classified by Stuckey (1962) into three groups: phenols, amines, and aminophenols. In general, they are structurally similar in that they contain unsaturated benzene rings plus either hydroxy or amino groups. Some are also effective polymerization inhibitors and others retard degradation of polymeric systems by ozone.

Phenols are commonly used antioxidants when low color, low toxicity, or a combination of these properties is more important than extreme potency. Most natural and synthetic food grade antioxidants belong to the phenolic class of compounds (Dugan, 1960).

Antioxidants with amino or diamino groups attached to unsaturated benzene rings are usually extremely potent, often quite toxic and generally form intense colors when oxidized or when they react with metals to form salts. They are usually quite stable to heat and caustic extraction. An example is diphenylamine which is widely used in rubber industry (Dugan, 1960; Sherwin, 1968).

Aminophenol antioxidants have both amino and phenolic groups as potential sites of antioxidant activity. They are used in the prevention of gum formation in gasoline (Lundberg, 1962a).

The unsaturated benzene rings found in the antioxidants are responsible for the stabilization of free radicals through the formation of stable resonance hybrids after hydrogen is donated to free radicals during oxidation (Figure 6).

Most Commonly Used Food Antioxidants

At present, the commonly used food antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl galate (PG), tocopherols, and hydroquinone. Structures of the first three are represented in Figure 7. Among the metal sequestering agents, citric acid is used most commonly (Sherwin, 1972).

Due to the occurrence of synergism among these antioxidants and due to regulations regarding the amount that may be added to foods (U.S.D.A., 1960), they are used most commonly in mixtures rather than separately (Sherwin, 1972).

The development of new antioxidants has been continuously undertaken by many workers. Olcott (1974) reported a new antioxidant being widely used in Europe (Figure 8). He stated that the compound was excellent for fats and oils and was not absorbed in the gastrointestinal tract as most others are. The latter aspect is undoubtedly very promising since such a compound could be used without limitation and could possibly solve tough problems of

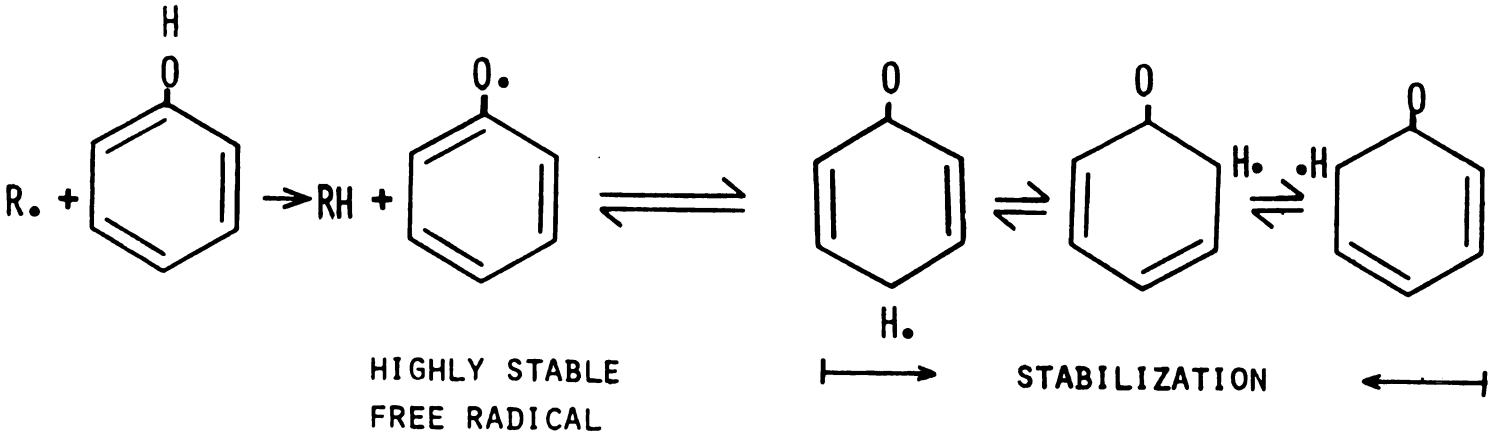
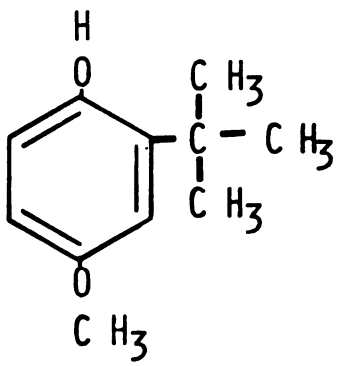
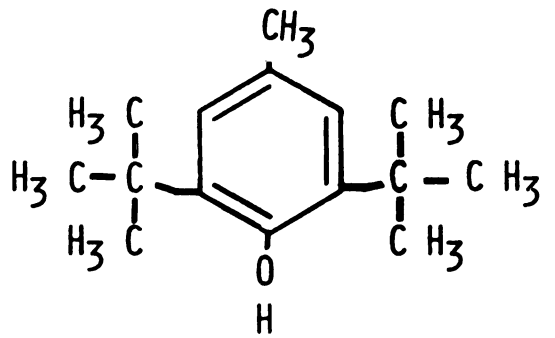


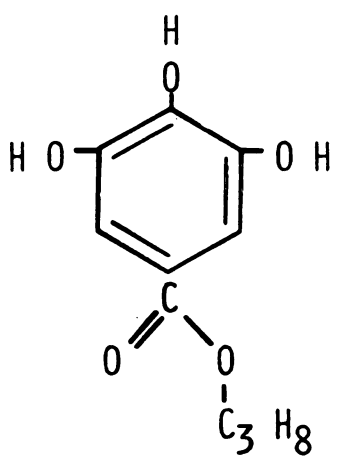
Figure 6.--Stable Resonance Hybrids Formed by Phenolic Antioxidants.



BUTYLATED HYDROXYANISOLE



BUTYLATED HYDROXYTOLUENE



PROPYLGALLATE

Figure 7.--Structure of Most Common Food Antioxidants.

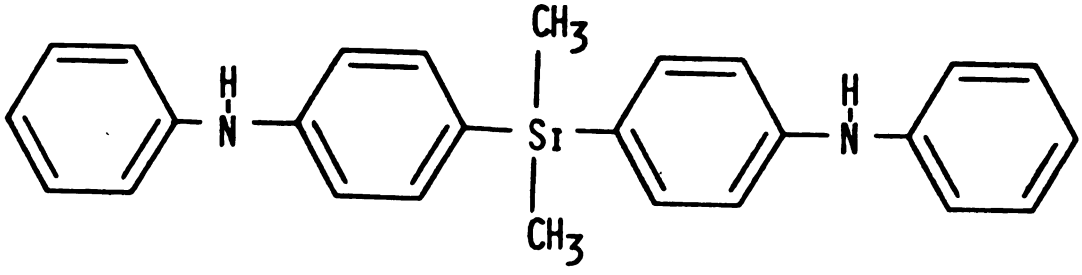


Figure 8.--A New Antioxidant in Use in Europe.

lipid oxidation in many foods that are still unresolved.

Silicones

Silicone compounds are being used more and more for their antioxidant and their anti-foaming action (Weiss, 1970; Freeman et al., 1973). When added to frying fats and shortenings, they result in improved stability. The action of such polymethyl siloxomes, more simply silicones, supress foaming, decrease oxygen contact, and supress build-up of foam promoting oxidation products in the fats or oils being heated. The mechanisms for this supression have been presented and discussed by Freeman et al. (1973). Recommended usage levels for silicones are in the range of 0.5 to 3.0 ppm (Weiss, 1970; Freeman et al., 1973).

In addition to citric acid as a chelating agent, especially in high moisture and fatty foods, ascorbic acid has been utilized. Ascorbic acid will chelate metal ions; however, the mechanism of antioxidant activity is more complex in high moisture systems (Labuza, 1971). An oxygen scavenger mechanism attributed to ascorbic acid was proposed by Cort (1974) as an explanation for increased stability of oil systems containing ascorbic acid. Ascorbic acid has been shown to have pro-oxidant activity in meat (Love and Pearson, 1971; Benedict et al., 1975). Citric acid and ascorbic acid have beneficial

synergistic effects with Type I antioxidants (Dugan, 1960). In addition to citric and ascorbic acid, polyphosphates are also known to serve as metal chelators.

Selection and Use of Antioxidants

The selection and use of antioxidants and/or mixtures should be done carefully and with consideration of the conditions surrounding the manufacture, handling, and use of the food product to be treated. According to Sherwin (1972), the major selection criteria to be considered include: antioxidant potency, solubility or dispersability, discoloration tendencies, food pH, processing, antioxidant odor and flavor, mode of application, and future trends in industry for that product.

Some important points to remember in using antioxidants are the necessity of early addition, uniform and complete distribution of the antioxidants in the food being treated. It is important to observe these points due to the chain nature of the antioxidation of lipids, the unrestorability of oxidized fats and oils, and the small amounts of antioxidants normally used (Lea, 1960; Sherwin, 1960). The reduction of oxygen incorporation and minimization of trace metals present are also important.

The relative efficiencies of a series of antioxidants can be altered considerably by small changes in the

composition of substrate, or even in the temperature of oxidation (Lea, 1960). The difference in the activity of an antioxidant from one complex food to another can be very large (Lea, 1962).

After the above considerations and the lack of data regarding the use of antioxidants for a particular product, one may eventually decide upon experimentation to devise a new formulation or determine among various possible ones which product gives the best results. However, with a large number of antioxidants available, one may have too many combinations for a practical experiment even though it could give the best solution to the problems. This problem can be solved partially through elimination of poor formulations. The four most commonly and widely used antioxidants and their characteristics were presented in several reports and reviews available in literature (Dutton et al., 1948; Kraybill et al., 1954; Gearhart and Stuckey, 1955; Sims and Hilfman, 1956; Cooney et al., 1958; Lea, 1960, 1962; Dugan, 1960; Lundberg, 1961, 1962a, 1962b; Stuckey, 1962; Weiss, 1970; Jacobsen and Koehler, 1970; Mickelberry, 1970; Sherwin, 1972; Joint FAO/WHO Expert Committee, 1972; Bishov and Hekick, 1972; Klinger, 1974). The following factors are important when selecting an antioxidant for a particular food:

1. Trace metals such as Fe, Cu, Co, and Ni are expected to be present in foods and they catalyze fatty acid oxidation.
2. Citric acid and ascorbic acid can chelate metals and show some synergistic effects on BHA and BHT.
3. BHA and BHT show similar mode of action; sometimes BHT may be slightly more potent than BHA.
4. BHA and BHT show good carry-through properties during baking, cooking, and frying.
5. BHA and BHT show good synergism when used in 1:1 proportions.
6. Propylgallate shows synergism with BHA and not BHT, does not have good carry-through property and may develop undesirable color when used in the absence of metal chelators.

Extraction of Tissue Lipids

Qualitative extractions of tissue lipids can be achieved by using a combination of polar and nonpolar solvents. Nelson (1975) suggested hydrolysis before extraction to facilitate separation of protein-bound lipids and allow free access to nonpolar solvents. Folch et al. (1957) used a 2:1 chloroform-methanol mixture to extract tissue lipids. Bligh and Dyer (1959) used a

mixture of chloroform-methanol and water as lipid extracting solvents. A chloroform-methanol mixture has been suggested as a good lipid extracting solvent by others including Ostrander and Dugan (1961) and Radin (1969). Based on the effect of various combinations of solvents on extraction of total lipids, fatty acids, and triglycerides, Sheppard et al. (1974) found that digestion with 4N HCl followed by diethyl ether extraction or 2:1 chloroform:methanol extraction were satisfactory methods for lipid extraction.

Separation and Fractionation of Phospholipids

Silicic acid column chromatography is the most popular and efficient method used to separate polar lipids from tissue lipids of animal origin (Hanahan et al., 1957; O'Brien and Benson, 1964; Kata et al., 1966; Peng and Dugan, 1965; and Nelson, 1975). Usually neutral lipids, glycosphyngolipids and phospholipids can be separated by this procedure. The treating of silicic acid with isopropanol-KOH to separate free fatty acids has also been reported (McCarthy and Dutchie, 1962).

Thin layer chromatography (TLC) has become a major procedure for lipid fractionation in the past two decades. It has advantages over silic acid column separation with respect to resolution, speed of separation, and simplicity of procedure (Nelson, 1975). Silicic acid column

chromatography can be very useful when large amounts of the fractions are required for further analyses. Complex mixtures of lipids can be successfully and reliably separated using two-dimensional TLC on silica gel plates. Skipski et al. (1962) separated phospholipids and cerebrosides on silica gel plates using a mixture of chloroform, methanol, acetic acid, and water as developing solvents. Parker and Peterson (1965) found a special washed silica gel H plate and a solvent mixture of chloroform, methanol, acetic acid and water to be good for microfractionation of phospholipids. The two dimensional TLC method of Rouser et al. (1966) with chloroform, methanol, water, n-butanol acetic acid and aqueous ammonia for first dimension and chloroform, acetone, methanol acetic acid, and water for second dimension is a popular and successful method for phospholipid fractionation. Following fractionation of phospholipids, further estimations have been achieved by gravimetric, colorimetric, and titrimetric analyses. Colorimetric methods are generally accepted because of high reproducability of results. Common methods include those of Morrison (1964), Parker and Peterson (1965), and Rouser et al. (1966).

Gas Liquid Chromatography (GLC)

Purified total lipids or fractions belonging to various classes of lipids need to be converted to a volatile form before GLC analyses. This may be achieved by converting lipids directly into volatile forms or by hydrolysis of the lipids, and converting fatty acids separated into volatile forms (Metcalfe et al., 1966). Reagents and methods depend on the type of compound being analyzed. For conversion of fatty acids with less than eight carbons in the chain, BF_3-butanol reagent is preferred, while fatty acids with more than eight carbons in the chain can be converted using BF_3-methanol or BCl_3-methanol (Metcalfe et al., 1966). Soponification before esterification often gives best results. Some of the common procedures in general acceptance and wide use are those methods of fatty acid methyl ester preparations reported by Morrison and Smith (1964), McGinnis and Dugan (1965), and Metcalfe et al. (1966).

Since the introduction of GLC as a technique for separation of carboxylic acids (James and Martin, 1952), it has been successfully used as a major technique for separation of lipids, alcohols, hydrocarbons, pesticides, and amino acids.

Efficient and normal separation of a chemical mixture can be accomplished by use of a liquid phase similar in

chemical structure to that of the mixture (Orr and Callen, 1958). They used a polyester type of liquid phase for successful separation of polyunsaturated fatty acids. Some widely used liquid phases are adipate and succinate polyesters of diethylene glycol. Silicone polymers of type SE-30 especially for high temperature separation of natural triglycerides has been recommended by Kuksis (1965).

Factors such as type and amount of liquid phase, temperature, gas flow rates, type of compound being analyzed and sample size have influenced efficiency of separation and resolution of chromatograms (Mehlenbackner, 1960; Seino et al., 1973).

Qualitative identification of the various peaks on fatty acid GLC chromatograms can be accomplished by using retention value or by using the plot of log retention time of standard fatty acids against fatty acid chain length. This method described by James (1960) results in a linear straight line relationship for homologus series, thus is very useful for qualitative identification of fatty acids.

METHODS AND MATERIALS

Processing and Treatment of Fish Samples

Fish Samples

Carp (Cyprinous carpio) samples used in this study were obtained from a commercial fishing plant, Bay Port Fish Co., Bay Port, Michigan. All fish were harvested from Lake Huron. The fresh fish were transported to MSU Meat Laboratories, ice packed in cardboard boxes. Overall processing and treatments were completed within 36 to 48 hours of initial harvest. The fish used were 39 to 62 cm long and weighed from 2.50 to 4.10 kg each.

Mechanically Deboned Carp

About 500 kg of carp were washed thoroughly in cold running water, weighed, and manually deheaded, gutted and split dorsoventrally parallel to the backbone. The split halves were washed in cold running water, care being taken to remove peritoneal membranes, connective tissues in the abdominal cavity and kidney tissue, and immediately layered with crushed ice before deboning.

A Bibun model SDS13 deboner (Bibun Co., Fukuyama Kovoshima, Japan) was used to debone the fish. This is a moving belt type machine equipped with 3 mm hexogonal

perforations in the drum. Dressed fish were fed into the machine so that the flesh side was next to the drum perforations and the skin side was next to the belt. The meat was pressed through the drum perforations to the inside while bone, skin, scales, and some connective tissue pass outside the drum. The mince coming out of the inner surface of the drum was collected in rectangular pans and stored at $2^{o}C$ for further treatments. All treatments were completed within 6 to 8 hours on the same day.

Hand Filleted Carp (with skin)

A total weight of 400 kg carp used in this study was ice packed in waxed cardboard boxes and transported to the University Meat Laboratories. The fish were thoroughly washed in cold water ($2^{o}C$) to remove slime and were weighed. After weighing, fish were manually deheaded, gutted, and split dorsoventrally so that two fillets and a vertebral column were separated. The fillets were washed thoroughly using running cold water, and care was exercised to remove peritoneal membranes, connective tissues in the abdominal cavity, kidney and other organ tissues. The washed fillets were weighed and stored in layers of crushed ice for further treatment.

Hand Filleted Carp (without skin)

The skins were removed from one sample of the fillets by hand using a sharp knife, washed, weighed and stored in layers of crushed ice for further treatment.

Antioxidants and Chelators Used

Only legally permitted antioxidants were used, except for one treatment consisting of ethylene diamine tetra-acetic acid (EDTA). Following are the antioxidants and chelators used in this study:

1. Tenox 2® - Eastman Kodak Co., Rochester, N.Y.
2. Tenox BHA® (Butylated hydroxy anisole) - Eastman Kodak Co., Rochester, N.Y.
3. Tenox BHT® (Butylated hydroxy toluene) - Eastman Kodak Co., Rochester, N.Y.
4. Propylgallate (PG) - Eastman Kodak Co., Rochester, N.Y.
5. L+ ascorbic acid (AA) - Eastman Kodak Co., Rochester, N.Y.
6. Citric acid (CA) - Eastman Kodak Co., Rochester, N.Y.
7. EDTA - Mallinckrodt, St. Louis, MO.
8. Freezgard (Formula FP-88E) - Stauffer Chemical Co., Westport, CT.

Tenox 2® is a commercial food grade antioxidant mixture. It consists of 20 percent BHA, 6 percent propyl-gallate, 4 percent citric acid, and 70 percent propylene glycol.

Freezgard® (Formula FP-88E) is a mixture of NaCl, Na tripolyphosphate and Na erythorbate with a suggested use of 0.18 percent based on flesh weight.

All of the above antioxidants were incorporated at the rate of 0.01 percent for a single antioxidant or 0.02 percent for a combination of antioxidants (with no single antioxidant exceeding 0.01 percent) based on the total fat content, except for Freezgard®, which was used 0.18 percent based on flesh weight. The quality and quantity of antioxidants used are indicated in Table 1.

Incorporation of Antioxidants into Mechanically Deboned Carp Flesh

The antioxidants which were not soluble in water were dissolved in propylene glycol to make a 10 percent solution before appropriate addition to the sample of the deboned carp flesh as shown in Table 1. Minced fish was mixed in a Hobart Kitchen Aid food mixer equipped with a paddle (The Hobart Mfg., Co., Troy, OH). The stainless steel mixer bowl was covered with a water repellant wax coated cardboard during four minutes of mixing at medium speed to allow the addition of N_2 during

Table 1. Antioxidant treatments used for mechanically deboned carp flesh[1].

Antioxidant Treatment	Number and Treatment Concentration														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tenox 2®	--	--	--	--	--	--	--	.02	--	--	--	--	--	--	--
Freezgard®	--	--	--	--	--	--	.18	--	--	--	--	--	--	--	--
BHA	--	--	--	--	--	.01	--	--	.01	.0075	.008	.006	.008	.006	--
BHT	--	--	--	--	.01	--	--	--	.01	.0075	--	.006	--	.006	--
Propyl gallate (PG)	--	--	--	.01	--	--	--	--	--	--	.006	.004	.006	.004	--
Citric acid (CA)	--	--	.01	--	--	--	--	--	--	.0050	.006	.004	--	--	--
Ascorbic acid (AA)	--	.01	--	--	--	--	--	--	--	--	--	--	.006	.004	--
EDTA	.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--

[1]All values are percent based on total fat weight, except Freezgard which is based on total flesh weight.

the mixing operation. Oxidation was minimized by maintaining low product temperature (8-10°C) and injecting nitrogen into the mixer bowl during mixing.

Packaging of Mechanically Deboned Carp Flesh

140 g samples of each fish product prepared as above was packaged in a #13 IKD plastic bag (17x10 cm) in duplicate (140 g per package). One set of duplicate samples for each period of analysis from each treatment was heat sealed without air evacuation from the plastic pouch, a second set of duplicate samples was vacuum sealed, and a third set of duplicate samples was evacuated, backflushed with 25 psi nitrogen and heat sealed.

Freezing and Storage of Mechanically Deboned Carp Flesh

All samples were frozen in air blast freezers at -26°C for 48 hours and then packaged in wax coated cardboard boxes and stored for 11 months at -20°C. Samples were removed for further analysis as needed.

Incorporation of Antioxidants into Hand Filleted Carp (with and without Skin)

All antioxidants were prepared as 4 percent solutions using propylene glycol and water or propylene glycol alone and mixed to obtain appropriate combinations and concentrations (Table 2). To 25 ml of each antioxidant mixture 100 mg TWEEN-20C polyoxyethylene sorbitan monolaurate was added to facilitate dispersion of the antioxidants. As

Table 2. Antioxidant treatment used for hand filleted carp with and without skin[1].

Antioxidant	Antioxidal Concentration									
	Fillet With Skin					Fillet Without Skin				
	1	2	3	4	5	1	2	3	4	5
Tenox 2®	.02	--	--	--	--	.02	--	--	--	--
Freezgard®	--	.18	--	--	--	--	.18	--	--	--
BHA	--	--	.006	.006	--	--	--	.006	.006	--
BHT	--	--	.006	.006	--	--	--	.006	.006	--
Propylgallate (PG)	--	--	.004	.004	--	--	--	.006	.004	--
Citric acid (CA)	--	--	.004	--	--	--	--	.004	--	--
Ascorbic acid (AA)	--	--	--	.004	--	--	--	--	.004	--

[1]All values were calculated as a percentage based on total fat content, except freezgard which was based on total flesh weight.

required the mixture was diluted and spread uniformly onto the surface of each fillet before freezing. The fillets were frozen in a blast air freezer at -26°C, wrapped individually in a brown paper wrap, packaged in water repellent wax-coated cardboard boxes and stored in a freezer maintained at -20°C for periods up to 11 months. Samples were removed for further analyses as needed.

Sampling of Carp for Seasonal Variation Analyses

About the 15th of each month from May, 1980, to April, 1981, 6 to 8 female carp 60 to 70 cm long were harvested from Lake Huron, ice packed and transported to the University Laboratory. Length, from tip of snout to end of upper dorsal fin, width behind head and weight were recorded. The flesh was separated by hand, ground and representative samples were analyzed for proximate composition, fatty acid profile and total cholesterol content.

Presentation of Samples for Further Analyses

Frozen samples of mechanically deboned carp for further analyses were partially thawed, cut into 1 cm cubes and a random sample selected for approximate analysis.

Frozen fillets for each evaluation period were thawed and skin and bone removed. The deboned carp flesh

was cut into strips 20 to 25 cm long and ground using a Hobart Kitchen Aid K5-A food mixer equipped with a grinding attachment and fitted with a 3 mm perforated plate (The Hobart Mfg. Co., Troy, OH). All samples were placed in plastic bags and stored on ice until evaluation. A representative sample was obtained after the ground tissue was mixed by hand. TBA analyses and lipid extractions were performed immediately after sampling to minimize lipid oxidation.

Materials for Chromatographic Analyses

Stainless steel columns (0.32 cm x 1.83 m) packed with 10 percent DEGS-PS (Diethylene glycol succinate-phosphoric acid) on 80-100 mesh Supelcoport were obtained from Supelco Inc. The columns were conditioned at 60°C for 5 hours and then temperature programmed from 50°C to 195°C at 2°C/min followed by holding at 195°C for 24 to 36 hours, during which time 20 ml/min nitrogen flow rate was maintained. These columns were later used for fatty acid analyses.

Silane treated glass columns (0.64 cm x 1.83 m) packed with 3 percent OV-17 on 100-200 mesh gas-chrom Q, was used for steroid analyses. These columns were obtained from Applied Science Laboratories, Inc. Columns were conditioned using a nitrogen flow rate of 40 ml/min and a programmed temperature of 50-300°C at 2°C/min,

followed by 24 - 36 hours holding. Thin layer silica gel (0.25 mm) glass plates with Redi-coat G, H and 2-D were purchased from Supelco Inc. Thicker 0.4 mm - 0.55 mm Silica gel plates were prepared in the laboratory using a template. All plates were activated before use by heating to a temperature of 120^{o}C for 40 min.

Silicic acid was obtained from Mallinckrodt Co., Inc. The fines were removed by several washes with deionized distilled water followed by washing in anhydrous methanol and activated by heating to 110^{o}C for 24 hours before packing into glass columns. Analytical grade solvents were used after they were glass distilled. When required, chloroform was stored using 0.25 percent methanol. All chemicals and solvents were purchased from Fisher Scientific Company.

All chromatographic standards including fatty acid methyl esters, cholesterol, 5-cholestene were obtained either from Applied Science Laboratories, Inc., or Supelco Inc.

The antioxidants used were purchased from Kodak-Eastman Organic Chemicals. Potential aldehydes and peroxides in the alcohols used in the extraction of lipids were removed by distillation over potassium hydroxide pellets. The redistilled alcohols were stored in brown bottles and used in less than two months.

Chlorinated Solvents

Chloroform in metal cans, when used in this experiment, was purified from possible phosgene ($COCl_2$) contamination by washing with water, drying over calcium chloride, and then distilling. To inhibit further production of phosgene, about 0.25 percent methanol was added. However, fresh commercial reagent grade glass distilled chloroform with 0.25 percent methanol was used directly.

Petroleum Ether and Diethyl Ether

Fresh bottles of reagent grade petroleum ether and diethyl ether were redistilled after testing for presence of peroxide as follows: 2 ml of solvent with 1 ml of freshly prepared 10 percent KI solution were shaken and then a drop of starch indicator solution was added. A rapid development of blue color indicated presence of peroxides.

Analytical Method

Moisture

The A.O.A.C. (1975, 25.003b) procedure for determination of moisture was used throughout the experiment. Triplicate 5 g samples were weighed into previously heated, cooled, tared aluminum weighing pans and stored in a desiccator. The samples were dried overnight (18 hours) at 100°C in a forced-air oven. The weight lost

during drying and cooling was expressed as moisture content.

$$\% \text{ moisture} = \frac{\text{weight of moisture (g)}}{\text{weight of initial sample (g)}} \times 100$$

Total Fat

Total solids, after moisture determinations, were used for total fat determination. Goldfisch ether fat extraction method (A.O.A.C., 1975, 24.005b) was used. Known weights of dry samples were extracted continuously for 2 1/2 hours, using anhydrous ethyl ether. The lipid extract was then separated from ether by distillation and residual ether was evaporated by drying at 100°C for 30 minutes. The weight of cooled ether extracted material was used to calculate the total fat content based on fresh weight of the sample, using the equation.

$$\% \text{ fat} = \frac{\text{weight of dried ether extract (g)}}{\text{weight of initial sample (g)}} \times 100$$

Protein

The semi-micro Kjeldahl procedure was used to determine the total crude protein content (A.O.A.C., 1975, 23.009). Triplicate 0.5 g samples were digested by boiling with 1 g sodium sulfate, 7 ml concentrated sulfuric acid, and 1 ml of 10 percent (w/v) copper sulfate solution. A clear pale green color of the boiling

solution indicated completion of digestion (approximately 2 1/2 hours digestion period). The digested sample was neutralized with 50 percent sodium hydroxide and steam distilled to collect 30 ml distillate in an Erlenmeyer flask containing 10 ml (w/v) boric acid. The distillate was back titrated with standardized 0.1 N sulfuric acid to a colorless bromocresol green end point. Percent protein was calculated as follows:

$$\% \text{ protein} = \frac{(\text{ml of } H_2SO_4 \times \text{N of } H_2SO_4) \times (0.014) \times (6.25)}{\text{weight of initial sample (g)}} \times 100$$

Ash

Total ash content was determined using the A.O.A.C. (1975, 20.012) method. Triplicate 5 g samples of fresh product were weighed into previously ashed, overnight dried (100°C), cooled and tared crucibles. These crucibles were heated in a muffle furnace at 525°C until a uniform white ash was obtained (overnight). The ashed crucibles were cooled in a dessicator and weighed. The percent ash was calculated as follows:

$$\% \text{ ash} = \frac{\text{weight of ash residue (g)}}{\text{weight of initial sample (g)}} \times 100$$

Extraction of Total Lipids

Total lipids from all samples used for further lipid analyses were extracted by the method described by Folch et al. (1957), with a slight modification. A known quantity of ground carp muscle was homogenized at high speed with a 2:1 chloroform:methanol solution (about 4 times the sample weight). The extracting solvent and the tissue residue were then transferred to a Buchner funnel fitted with #1 Whatman filter paper and filtered under slight vacuum. The residue with filter paper was reextracted with 4 more volumes of 2:1 chloroform:methanol. The extracting solvent was filtered and both filtrates were collected in a separatory funnel. The crude extract was washed with 0.2 volume of 0.74 percent potassium chloride solution and allowed to stand at -18^{o}C overnight to facilitate separation and minimize oxidation of the lipids. The chloroform layer was collected by passing over anhydrous sodium sulfate into a glass stoppered round bottom flask. The water layer was washed with two 25 ml portions of chloroform and combined with the chloroform layer collected earlier. The solvent was evaporated at room temperature by using a rotary vacuum evaporator (Rinco Instrument Co.). Removal of traces of chloroform was facilitated by passing a stream of dry N_2 over the extracted lipid. The total lipid was transferred under N_2 to vials with air tight teflon-lined screw caps

and stored at -18°C for further analyses.

Separation of Neutral and Phospholipids

Preparation of Silicic Acid Columns

A silicic acid column was prepared according to the method of Rouser et al. (1966). A 25 g sample of washed and activated silicic acid was dispersed in 75 ml chloroform and poured into a 1.5 x 30 cm glass column. Stopcock of the column was opened and the column was gently tapped to dislodge air bubbles and to aid settling. The solvent level was allowed to drop to the top of the silicic acid level in the column, the bed was washed with 2 column volumes (ea 45 ml) of chloroform.

Application of Sample

With the solvent level at the top of the silicic acid, 200-250 mg of total lipids in 3 ml chloroform were added carefully down the sides of the column. Quantitative transfer of sample was ensured using two, 2 ml portions of chloroform. Elution with chloroform was then carried out by connecting the column to a chloroform reservoir. Elution was performed at 2 ml/min with slight pressure using a stream of N_2 to collect 250 ml eluents containing neutral lipids. To ensure completion of elution of neutral lipid, a negative Salkowski test was used as an indicator. In this test 1 ml of eluant

was added to 1 ml of concentrated sulfuric acid. A characteristic brown to yellow band indicated a positive result. A negative result indicated completion of the elution. Following chloroform elution the column was washed with 500 ml of acetone to remove pigments and then phospholipids were collected from the column using 200 ml of methanol. A negative ninhydrin test was used to check the completeness of recovery (the ninhydrin test was done by adding ninhydrin to 2 ml methanol eluant in a vial and covering it with a screw cap. On boiling, development of purple color indicated a positive test while no color development indicated a negative test.)

Purity of phospholipids evaluated by TLC on a 0.25 mm thick silica gel G plate and a solvent system consisting of petroleum ether, ethyl ether and acetic acid (90:10:1 by volume). The purity of neutral lipids was checked by TLC on a silica gel G plates with a solvent system of chloroform, methanol and water (65:25:4 by volume).

The solvents from separated neutral and phospholipids were evaporated on a Rinco rotary vacuum evaporator. Most traces of solvent were removed under a stream of dry N_2. The fractions were dissolved in known amounts of chloroform and quantified gravimetrically by using a small portion of standard solution. The fractions were stored under N_2 in vials with Teflon lined screw caps at $-18^{o}C$ for further analyses.

Classification of Phospholipids

The analysis procedure described by Rouser et al. (1966) was used to classify phospholipids. Phospholipid solutions obtained from silicic acid column chromatography were applied onto a 0.5 mm thick silica gel G plate, 2 cm from the bottom as several spots 1 cm apart. A 20 percent phospholipid solution in chloroform:methanol (2:1) was used for spotting with a calibrated capillary (Scientific Products, Romulus, MI) under a stream of nitrogen. A spot with a mixture of a standard solution of phospholipids was applied at one end. Immediately after spotting, plates were transferred to TLC chambers lined on all sides with a filter paper saturated with chromatographic solvent. The solvent stood to a height of about 1 cm at the bottom. In the first dimension the plates were developed with chloroform-methanol-28 percent aqueous ammonia (65:25:15). The chromatogram was dried under a stream of dry N_2 for 6 to 8 minutes and then developed in a second dimension using a mixture of chloroform, acetone, methanol, acetic acid and water (3:4:1:1:0.5). Dried chromatograms were exposed to iodine vapor or sprayed with 0.5 percent iodine in a methanol solution to visualize the spots of separated phospholipids. The spots were marked and iodine was allowed to sublime under a stream of nitrogen. These phospholipid spots and a similar sized area on the plate adjacent to them were

scraped and transferred directly into micro Kjeldahl flasks. The spots adjacent to the phospholipid locations were used as blanks.

Phosphorous Determination

Phospholipid phosphorous was quantitatively analyzed for various phospholipid fractions using the method of Rouser et al. (1966). The scraped silica gel with the phospholipid was digested as follows. Perchloric acid (0.75 ml, 72 percent; distilled) was added to the phospholipid fractions separated as above and heated to boiling for 25 minutes over an electrically heated digestion rack. The flasks were shaken occasionally during digestion. After cooling, 5 ml of distilled water and one ml of 2.5 percent ammonium molybdate were added and mixed. One ml freshly prepared 10 percent ascorbic acid was added and the contents were quantitatively transferred to a centrifuge tube using two-1 ml rinses of distilled water. Color was developed by heating for 5 minutes in a steam heated water bath. Adsorbent was centrifuged down and the absorbance of the supernatent was noted at 820 nm using Bausch and Lomb Spectronic-20. The absorbance value obtained from the spot used as a blank was subtracted from the value from a sample to give corrected absorbance value of phosphorous content. Known amounts of phosphorous in the form of dried

disodiumhydrogenphosphate were spotted on similar silica gel plates and the spots were scraped and the transferred into a micro Kjeldahl flasks for phosphorous determination as above. These data were used to construct a standard curve for comparison.

Free Fatty Acid Separation

Free fatty acids were separated using an isopropanol-KOH treated silicic acic column washed with ethyl ether according to the procedure of McCarthy and Dutche (1962).

2-Thiobarbituric Acid (TBA) Analysis

TBA analyses were carried out according to the procedure of Tarladgis et al. (1960). Random 10 g samples were homogenized in a Virtis macro-homogenizer model 23 with 50 ml distilled water for 2 minutes. Homogenates were transferred with 47.5 ml distilled water to 500 ml boiling flasks and acidified with 2.5 ml hydrochloric acid:distilled water (1:2, v/v). Antifoam A spray (Dow Corning, Midland, MI) was used to prevent excessive foaming. Distillations were performed using a 300 mm Vigneux column attached to a 470 mm Leibig condenser with a 75^{o}elbow. Distillates of 50 ml each were collected from three distillations per sample within 10 to 15 minutes. All samples were heated uniformly. A TBA reagent (0.02 M 2-thiobarbituric acid) in 90 percent

redistilled acetic acid was prepared by dissolving 1.4415 g thiobarbituric acid (Eastman Organic Chemicals, Rochester, NY) in 50 ml distilled water and making up to 500 ml with distilled glacial acetic acid. A sonicator (Mettler Electronics Corp., Anaheim, CA) was used to aid in dissolving the TBA reagent. (When present, the removal of TBA reactive substances in commerical reagent grade glacial acetic acid was assured by reflexing the acetic acid with 2 g 2-thiobarbituric acid/liter, for three hours and redistilling it before use.)

Duplicate five ml samples of distillate were reacted with 5 ml of TBA reagent in capped culture tubes (200 mm x 25 ml) for 35 minutes in a boiling water bath and cooled in cold tap water for 10 minutes prior to spectrophotometric quantitation (Beckman DB Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA). Absorbance was read against a reagent blank at 538 nm. Reagent blanks were consistently run with each batch of distillate used for color development. TBA number (mg malonaldehyde/100 g sample) was calculated using 7.8 as a constant as described by Tarladgis et al. (1960).

Gas Liquid Chromatography of Fatty Acids

Methylation of lipid samples was carried out according to the method of Morrison and Smith (1964). The solvent was evaporated from each lipid sample with a

known amount of the fatty acid C21:0 added under a stream of dry nitrogen as an internal standard. One ml of 14 percent boron trifluoride was added under nitrogen and each tube was sealed with a teflon lined screw cap and heated for 10 minutes in a steam heated water bath. A similar procedure was followed for the preparation of neutral lipid methyl esters, with the addition of 0.2 ml of benzene and a heating time of 30 minutes. After heating, each sample was cooled to room temperature and methyl esters were extracted. Partitioning of methyl esters was achieved by separation in a mixture of petroleum ether and water (2:1). Triple extractions were performed. The extracted methyl esters were concentrated to a standard concentration and stored under nitrogen at -20^{o}C in 5 ml glass tubes with teflon lined screw caps for further analyses.

Gas liquid chromatographic analyses were performed using a Varian gas chromatograph (Model 3700) equipped with a CDS 111 integrator. Steel columns (0.32 x 182 cm) packed with 10 percent DEGS-PS on 80/100 Supelcoport (Supelco Inc., Bellefonte, PA) was used for fatty acid analysis. Nitrogen at 28 ml/min was used as the carrier gas. The hydrogen flame was supplied with 250 ml air and 12 ml hydrogen/minute. The injection port and detector were maintained at 240^{o} and 260^{o}C, respectively. The column temperature was programmed to remain at 145^{o}C for

10 minutes, followed by a 145 to 195°C increase at 1°C/ minute. Fatty acid C21:0 was used as internal standard for concentration calculation by the integrator.

Chromatograms of standard fatty acids and a plot of the logarithm of relative retention times (relative to methyl palmitate) versus the number of carbon atoms (where unsaturation results in a parallel line) was used for identification of all the peaks in the samples.

Cholesterol Analysis

Extraction of sterols was performed according to the method of Kovacs et al. (1979). Approximately 0.5 g of well-homogenized fish muscle was saponified directly in a tightly capped (teflon lined) 15 ml tube containing 1 ml 50 percent KOH and 4 ml 95 percent ethanol. The contents were boiled for 1 hour. Care was taken to see that the sample was immersed in saponifying solution. Tubes were then cooled to room temperature and 2.5 ml of water were added and unsaponifiables were extracted with four 5 ml portions of hexane. The combined extracts were evaporated to dryness under vacuum and redissolved in dimethyl formamide.

A standard amount of sterol was derivatised in a silane treated 15 ml centrifuge tube. Each solution sample was shaken vigorously in a test tube mixer with 0.2 ml hexamethyl disilazone and 0.1 ml tetramethyl

chlorosilane. After standing for 15 minutes, 1 ml of 5α-cholestane was added as internal standard (0.2 mg 5α-cholestane in n-heptane) and 10 ml of distilled water were added into the tube and shaken for one minute. The heptane layer was used for GLC analyses.

A cholesterol standard solution in dimethyl formamide was made to contain a stock solution of 2.0 mg/ml. Further dilutions of 0.05 to 1 mg/ml were prepared with 0.2 mg per ml 5α-cholestane in n-heptane and used as a standard curve. An F & M Model 810 dual column gas chromatograph was used to separate and quantify cholesterol using a 0.64 x 182 cm silane treated glass column packed with 3 percent OV-17 on 100-200 mesh gas-chrome Q. The column was operated at a programmed temperature from 240^{o}C to 275^{o}C at a rate of 6^{o}C per minute. The detector and injection ports were maintained at 275^{o}C. The nitrogen flow rate was 35 ml/min. The flow of air and hydrogen, respectively, were 350 ml/min and 35 ml/min. The peaks were identified by comparing their retention times with those of standards. The standard values obtained were used to quantify the cholesterol content of the sample.

Shear Values

Shear values were determined on fish gels. These gels were prepared by adding 3 percent NaCl to ground

fish muscle and mixing for 2 minutes in a paddle type mixer. The mixed tissue was molded to form blocks of 5 x 5 x 1.5 cm and heated in a convection oven to an internal end temperature of 74°C and cooled to room temperature. These blocks were weighed and sheared using a Texturometer (Food Tech. Corp., Rockville, MD) equipped with multi-blade compression cell and a 3,000 lbs transducer. The shear force was expressed as kg force/g sample (shear value).

Water Holding Capacity (WHC)

WHC was determined on fish gels prepared as above. Ten g of the fish gel were weighed into a pre-weighed centrifuge tube, one-third filled with glass beads. The tube was centrifuged for 20 minutes at 24,000 x g. The separated liquid was expressed as a percentage based on the original weight. This was used as an index of WHC.

Protein Solubility

Frozen muscle samples were coarsely powdered and mixed with chipped dry ice overnight and the dry ice was allowed to sublime before extraction.

The sarcoplasmic protein fraction was determined using the method of Helander (1957) as modified by Borton (1969). A 2 g sample of muscle was weighed into a 250 ml plastic centrifuge bottle equipped with a screw cap.

Fifty ml of precooled ($3^{o}C$) 0.015M phosphate buffer, pH 7.4 were added and stirred gently at $3^{o}C$ for 20 minutes. The mixture was centrifuged at 3500 x g for 25 minutes in a Sorvall RC2-B automatic refrigerated centrifuge. The supernatant was then passed through 8 layers of cheese cloth and collected in a 100 ml graduated cylinder. The residue was resuspended in 50 ml of 0.015M phosphate buffer and re-extracted, centrifuged, and filtered. The combined supernatant was designated as sarcoplasmic or water soluble protein fraction.

Myofibrillar protein was extracted from the residue after separation of the sarcoplasmic protein fraction, as follows. The residue was suspended in 50 ml of 1:1 M KCl in 0.1M phosphate buffer, pH 7.4. The mixture was stirred gently for one hour using a magnetic stirrer and centrifuged at 3500 x g for 25 minutes. The supernatant was collected and the residue re-extracted as described previously. The two extracts were combined and designated as total myofibrillar protein fraction.

Nonprotein nitrogen was determined by precipitating 15 ml of the sarcoplasmic protein fraction using 5 ml of 10 percent TCA solution and allowing the stirred mixture to stand for four hours. The supernatant was centrifuged (3000 x g for 25 minutes) and was designated as nonprotein nitrogen fraction.

Total nitrogen was determined from each fraction by semi-micro Kjeldahl procedure as described earlier.

Sensory Evaluation

Twelve panelists were selected at random from students, faculty, and staff of the Department of Food Science and Human Nutrition. Fish meat loaves were prepared using ground fish muscle from each treatment, with added onion soup mix, salt, and soy sauce. These products were sliced and coded with three digit random numbers and evaluated for rancid odor and rancid taste by panelists in segregated panel booths. A five-point hedonic scale was used (rancid = 5 and fresh = 1). A typical scoring form and instructions are enclosed in Appendix A.

Statistical Analyses

Data were analyzed on the CDC 6500 computer at Michigan State University. Correlations and a one-way analysis of variance was first performed on the data and then the data were treated as a split plot design with treatments as main effects and time as the sub effect. Duncan's New Multiple Range test (Steel and Torrie, 1960) was applied when analyses of variance data were significant, in order to detect the means with significant differences.

RESULTS AND DISCUSSION

Characteristics of Mechanically Deboned Carp

Yield

Minced carp yields were obtained by passing split carp once through a mechanical deboner.

Data presented in Table 3 indicates that a minced yield of 40 percent was obtained from carp by mechanical deboning compared to only 29 percent by hand deboning carp. The 40 percent yield obtained from carp by mechanical deboning was from 3 to 6 percent lower than that reported by Angel and Baker (1977) and Rippen (1981). Yield varied considerably depending on reproductive state of carp and male to female ratio in any processed batch since in gravid female fish, roe contributes significantly to the total weight. In this study it was observed that most of the fish were females in various gravid stages. Comparatively low yield of carp by mechanical deboning may also be due to the thick skin, extensive bone structure, and the large heads.

Proximate Composition

Proximate composition data are listed in Table 4. Mechanically deboned carp flesh contained higher total

Table 3. Effect of method of deboning on yield of minced carp.

Method of deboning	Fish portions	kg	Percent
MD[1]	whole	483.0	100.0
	mince	193.0	39.6
HD[2]	whole	87.0	100.0
	mince	24.8	28.5

[1]Mechanically deboned (from six batches).

[2]Hand deboned (n = 6 fish).

Table 4. Effect of method of deboning on proximate composition of minced carp.

Component	Method of Deboning	
	MD[1]	HD[2]
Moisture	69.95±0.64[a]	68.89±0.43[a]
Fat	15.56±0.98[a]	13.38±0.65[b]
Protein	13.53±0.45[a]	16.84±0.58[b]
Ash	0.96±0.04[a]	0.89±0.02[a]

[1]Mechanically deboned.

[2]Hand deboned.

Note: Values are mean percentages from 3 determinations.

Different superscripts in same row represents significant differences ($p<0.05$).

fat and lower protein and a slightly higher ash content than hand deboned carp. Little or no differences were observed in total moisture content between mechanically deboned and hand deboned carp flesh. In earlier studies reported by Webb et al. (1976), Crawford et al. (1972), and Rippen (1981), a higher moisture content was found in mechanically deboned flesh than hand deboned. This was not observed in this study. This difference might be influenced by the use of ice slush during storage of fish. Moisture could have been incorporated during the hand deboning and mincing process. The small difference in total ash content may be due to minimum incorporation of bone during single pass deboning. It is of importance to note that the deboned carp obtained by two or more passes through the deboner would result in a higher ash content due to incorporation of bone and also a higher moisture content (Wong et al., 1978). It may be noted that even if a single pass is followed, there may be variations in proximate composition based on the perforation size of the drum on the deboner.

The high fat content in mechanically deboned carp could be due to the depot fats from peritoneal cavity area and that on the dorsal region around the back bone. The high protein content in hand deboned carp could be due to the high proportion of lean meat in hand deboned carp.

Lipid Classes

Utilizing thin layer chromatographic procedures, the following six lipid classes were quantified from carp muscle. Phospholipids, monoglycerides, diglycerides, triglycerides, free fatty acids (FFA) and total cholesterol of which mono and diglyceride values are combined and listed in Table 5. A lower percentage of phospholipids and slightly higher percentage of triglycerides and cholesterol fractions were found in mechanically deboned minced carp than in hand deboned flesh. From the results expressed in mg of lipid fraction per 100 g of tissue, most of the high level of total lipids was due to triglycerides, mono and diglycerides and total cholesterol. This could be due to the incorporation of depot fats from skin, backbone and peritoneal cavity areas during mechanical deboning, while these depot fats are conveniently separated out during hand deboning. A slightly higher level of free fatty acid content was found in hand deboned minced carp than in mechanically deboned carp flesh. This could be due to the longer time involved in processing hand deboned flesh leading to enzymic hydrolysis of lipids.

Phospholipid Composition

Six different phospholipid fractions were identified and quantified from the polar lipid fraction of minced

Table 5. Effect of method of deboning on composition of different lipids in minced carp.

Lipid Class	Method of Deboning			
	MD[1]		HD[2]	
	mg/100 g tissue	Percent[3]	mg/100 g tissue	Percent[3]
Phospholipids	2200±79	14.14±0.51	2186±68	16.34±0.51
Mono and diglycerides	2080±70	13.3±0.45	1740±28	13.00±0.21
Triglycerides	10030±102*	64.46±0.66	8530±124	63.75±0.93
Free fatty acids (FFA)	420±23	2.70±0.15	444±37	3.32±0.28
Total cholesterol	680±21*	4.37±0.17*	350±28	2.62±0.21
Others[4]	150±16	0.43±0.13	130±17	0.97±0.10

[1]Mechanically deboned.

[2]Hand deboned.

[3]Percent based on total fat.

[4]Unidentified.

Note: All values are mean±SD.

*Indicates significant difference at $p<0.05$.

carp fat using TLC followed by phosphorous analysis. The composition and quantity of phospholipids are listed in Table 6. All minor fractions forming less than 1 percent of total phospholipids and those unidentified were quantified together and designated as "others". The various fractions consisted mainly of phosphatidylcholine phosphatidylethanolamine, spingomyelin, phosphatidyl-serine, phosphatidylinositol and cardiolipin. Phospha-tidylcholine and phosphatidylethanolamine accounted for about 60 percent of the total phospholipid content.

No significant differences were found in level of various phospholipid fractions between lipids of mechani-cally deboned and hand deboned minced carp could be identified. This may indicate that mechanical deboning does not affect the level of total phospholipids and their fractional components in spite of variations in the total lipid content.

Fatty Acid Composition

The fatty acid composition of phospholipids and neutral lipids from the total lipid extract from mechani-cally deboned and hand deboned minced carp are listed in Table 7. Twenty-four different fatty acids ranging from C14 to C22 were identified. Unsaturated fatty acids included fatty acids with one to six double bands. The fatty acid composition of phospholipids from mechanically

Table 6. Effect of method of deboning on composition of phospholipids in minced carp.

Phospholipid Class	Method of Deboning			
	MD[1] (mg/100 g tissue)	%	HD[2] (mg/100 g tissue)	%
Phosphatidylcholine	840±17	38.1	828±26	38.0
Phosphatidylethanolamine	504±21	22.9	497±19	22.8
Spingomyelin	237±16	10.8	235±18	10.8
Phosphatidylserine	240±13	10.9	229±11	10.5
Phosphatidylinositol	168±11	7.6	163±9	7.5
Cardiolipid	150±6	6.8	155±5	7.0
Others	65±9	2.9	73±8	3.4

[1]Mechanically deboned.

[2]Hand deboned.

Note: Values are means of 2 determinations, mg/100 g tissue±SD.

Percentages are based on total phospholipids.

Table 7. Fatty acid composition[1] of phospholipid and neutral lipid fractions from minced carp.

Fatty acids	Method of Deboning			
	MD[2]		HD[3]	
	Phospho-lipids	Neutral lipids	Phospho-lipids	Neutral lipids
14:0	26	491	26	409
15:0	8	67	7	57
16:0	382	1719	378	1357
17:0	4	86	4	309
18:0	219	376	229	89
20:0	4	104	4	110
Saturates	643	2843	648	2406
14:1	2	131	2	108
15:1	--	60	--	49
16:1	152	3254	156	2790
17:1	12	230	12	200
18:1	220	3150	212	2587
Monoenes	376	6825	382	5734
18:2	47	664	53	549
18:3 + 20:1	72	468	73	397
20:2	4	175	4	152
20:3	10	38	10	33
20:4 + 22:0	191	239	190	201
20:5 ω 6	236	96	229	79
20:5 ω 3	12	343	9	317
22:4	8	38	8	27
22:5 ω 6	32	12	28	10
22:5 ω 3	40	125	41	99
22:6	321	238	319	199
Polyenes	973	2436	974	2063

[1]Mean of duplicate samples.

[2]Mechanically deboned.

[3]Hand deboned.

Note: Values are mg fatty acid/100 g tissues.

deboned and hand deboned minced carp lipids did not differ. However, the ratio of saturated fatty acids to that of unsaturated fatty acids of phospholipids in mechanically deboned minced carp was 1:2.11 compared to 1:2.09 from hand deboned minced carp. While this ratio was very similar between two treatments, neutral fatty acids accounted for about 1:3.26 and 1:3.24 in mechanically deboned and hand deboned minced carp respectively. The polyunsaturated fatty acids formed a much higher percentage of phospholipids (about 49 percent) compared with about 20 percent of neutral lipids, However, the total amount (mg fatty acid/100 g tissue) of polyunsaturated fatty acids from neutral lipid would account for a substantially higher percentage of total polyunsaturated fatty acids in the whole system.

Carp lipids did not contain detectable levels of C15:1 fatty acids in the phospholipid fraction. C14:0, and C16:0 were the major saturated fatty acids, C16:1 and C18:1 were major monoenes and C18:2, C18:3, C20:3, C20:4, C20:5 ω 6, C20:5 ω 3 and C22:6 were the major polyenes.

The fatty acid composition of carp was similar to that reported by Mai and Kinsella (1979), except for minor concentration variations. This may be due to the variations in fatty acid composition of same species of fish from different areas, different seasons or different feed availability.

Protein Fractions

The level of nitrogen in the various fractions of the proteins from mechanically deboned and hand deboned minced carp are presented in Table 8. Higher percentages of sarcoplasmic and nonprotein nitrogen were found in mechanically deboned than in hand deboned carp. Stromal protein nitrogen was found to be much higher in hand deboned than mechanically deboned carp. These high values of sarcoplasmic and nonprotein nitrogen may be due to the incorporation of more free amino acids and proteins associated with the skin bone marrow and intra tissue fluids released during mechanical deboning (Webb et al., 1976). The lower stromal protein nitrogen levels may be due to the screening effect of mechanical deboning resulting in separation of collagen and fibrous connective tissue as reported by Satterlee et al. (1971) and Webb et al. (1976).

To develop the desired textural property of comminuted products using muscle proteins, an understanding of the solubility of individual proteins is important (Webb, 1974). However, the results based on protein fraction solubilities of fish muscle tissue are of no use in evaluating texture qualities (Webb, 1974; Webb et al., 1976). Basic information could be important in the development of comminuted products from mechanically deboned minced carp. For example, Carpenter and Saffle

Table 8. Effect of method of deboning on nitrogen levels of total and various extractable protein fractions from minced carp.

Method of Deboning	Protein fractions (mg/100 g tissue)									
	Total N		Sarcoplasmic N		Myofibrillar N		Nonprotein N		Stromal N	
	Mg	%[2]	Mg	%	Mg	%	Mg	%	Mg	%
Mechanically	2160±72	100	400±13	18.51±06	489±26	22.62±1	307±20	14.22±04	964±42	44.65±1.9
Hand	2690±93	100	441±15	16.36±06	619±18	23.02±07	308±27	11.46±1.0	1322±70	49.16±2.6

Note: Mean mg N/100 tissue ± SD from 4 determinations.

2 = Percent nitrogen based on Total N.

N = Nitrogen.

(1964) found a positive correlation between salt soluble proteins and emulsifying capacity of red meat muscle tissue. In this study no significant differences were observed in the myofibrillar protein fraction (salt soluble proteins) between mechanically deboned and hand deboned minced carp tissue.

Shear Value and Waterholding Capacity

The data presented in Figure 9 indicate that the fish gels prepared from mechanically deboned carp flesh had significantly lower shear values and firmness, as rated by a panel, compared to the fish gels prepared from hand deboned minced carp. The data on waterholding capacity, determined by the amount of liquid separated by centrifugation and panel scores for juiciness of fish gel prepared from mechanically deboned and hand deboned minced carp are presented in Figure 10. A high percentage of liquid separated from carp gel denotes a low water-holding capacity and vice versa. Although the water holding capacity of fish gel made from mechanically deboned minced carp was lower than from hand deboned carp (i.e., higher percent of extractable liquid), it was not significantly different from that of the gels prepared from hand deboned minced fish gel. A significant difference in juiciness was identified by the taste panel evaluation scores (2.86±0.4 and 3.8±0.5).

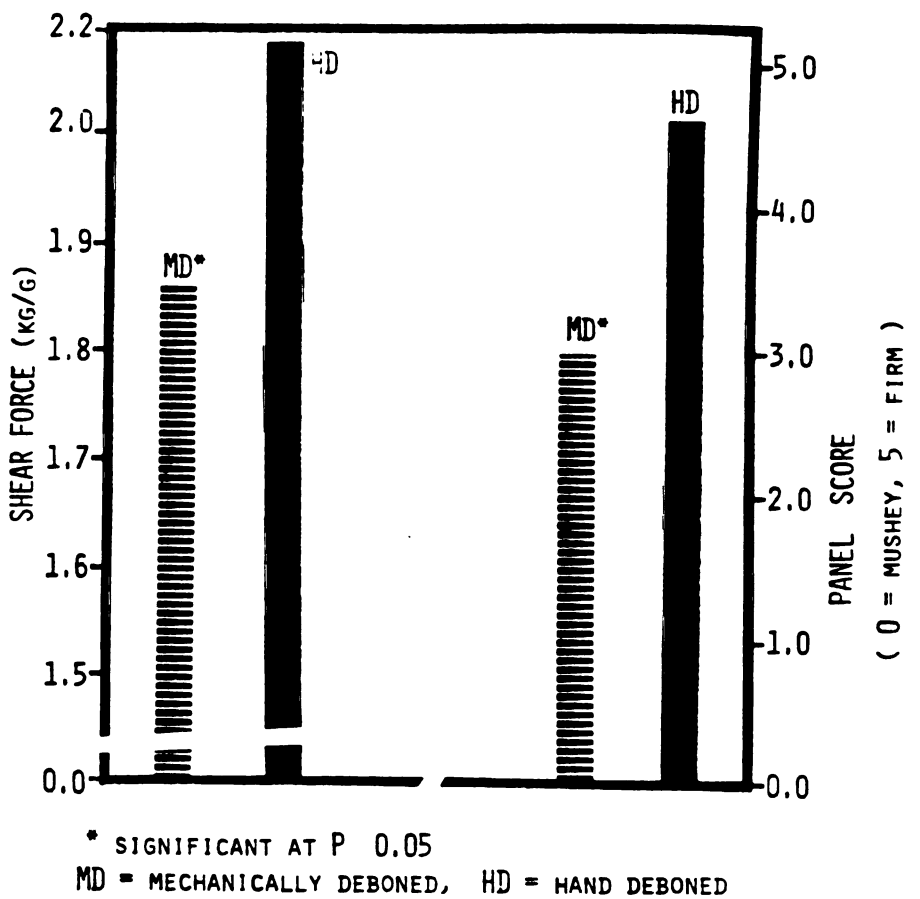


Figure 9.--Shear force values and panel sensory scores for firmness of fish gels prepared from hand and mechanically deboned minced carp.

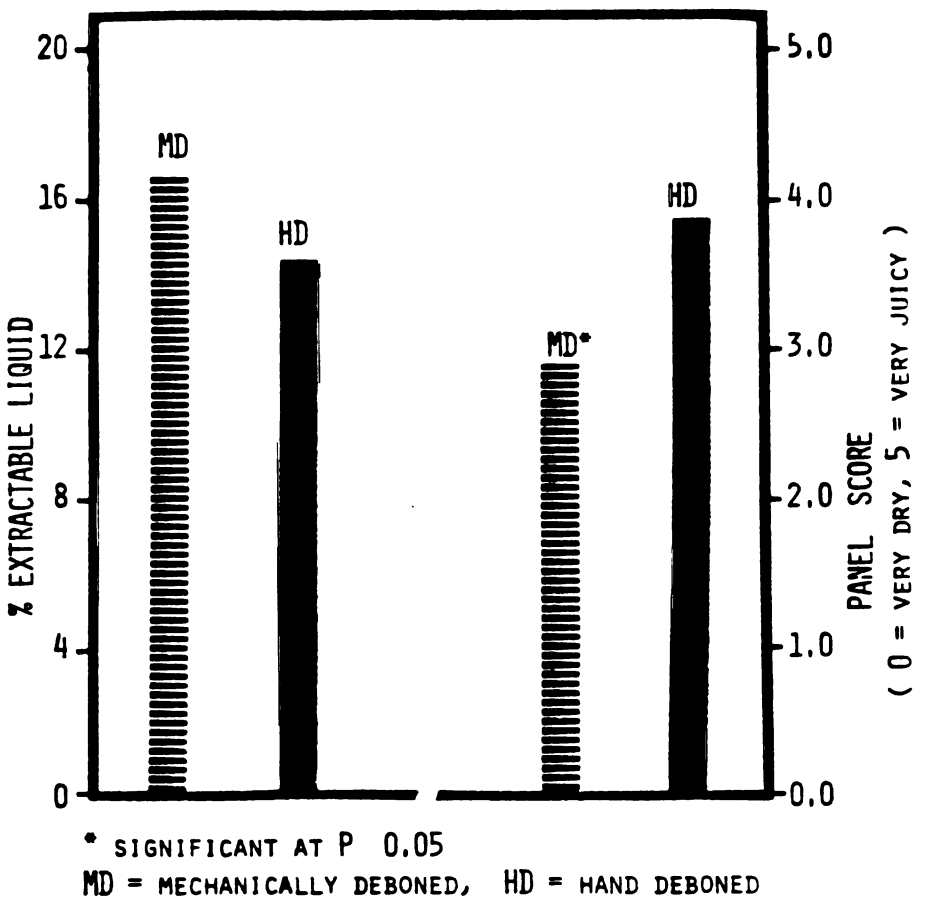


Figure 10.--Waterholding capacity and panel sensory score for juciness of fish gels from hand and mechanically deboned minced carp.

Lipids, their degradation products and their reactions with protein could be limiting factors affecting frozen storage life of fish fillets (Castell et al., 1966; Anderson and Steinberg, 1964; King et al., 1962; Hanson and Olley, 1965; Takama, 1974). The results from this study show that there are some additive effects when mechanically deboned minced fish is stored in the frozen state. Some adverse effects observed due partly to incorporation of high fat content from mechanically deboned flesh, were loss of solubility of some protein fractions, loss of waterholding capacity, and decrease in firmness of fish gels. To what extent these properties would effect the subsequent comminuted fish products were not determined, since product development from the frozen minced fish was not one of the objectives. In a similar study on the effect of mechanical deboning on fish flesh, Webb et al. (1976) concluded that a more firm product was obtained from hand deboned fish, probably due to the high soluble fraction in it. The shearing action during mechanical deboning could produce a stress upon myofibrillar proteins which may result in denaturation of fibrills and thereby low textural qualities of mechanically deboned fish.

Relative Stability of Mechanically Deboned Carp During Frozen Storage

During the mixing of antioxidants with mechanically deboned minced carp, the product temperature increased to 13±1°C. This temperature increase could be reduced by processing the fish in a controlled temperature room as in commercial seafood processing plants. Careful mixing was done to assure a homogenous distribution of antioxidants.

Preliminary Studies on TBA Test

A series of preliminary tests were conducted to determine the best conditions and procedures for determining TBA numbers. Preliminary trials indicated that a 35 minute heating period, followed by a 10 minute cooling period in the procedure suggested by Tarladgis *et al.* (1960) resulted in a more constant color formation in blanks than the 15 hour holding period at room temperature for color development as suggested by Tarladgis *et al.* (1964). Absorption spectrum of the pigments formed from TBA reaction in fish sample distillates are shown in Figure 11. A wave length of 538 nm was found to be most sensitive to the malonaldehyde-2-TBA complex pigment formation from minced carp flesh lipids. Distillation trials, using 1,1,3,3-tetra ethoxypropane (TEP) to determine average recovery and determination of

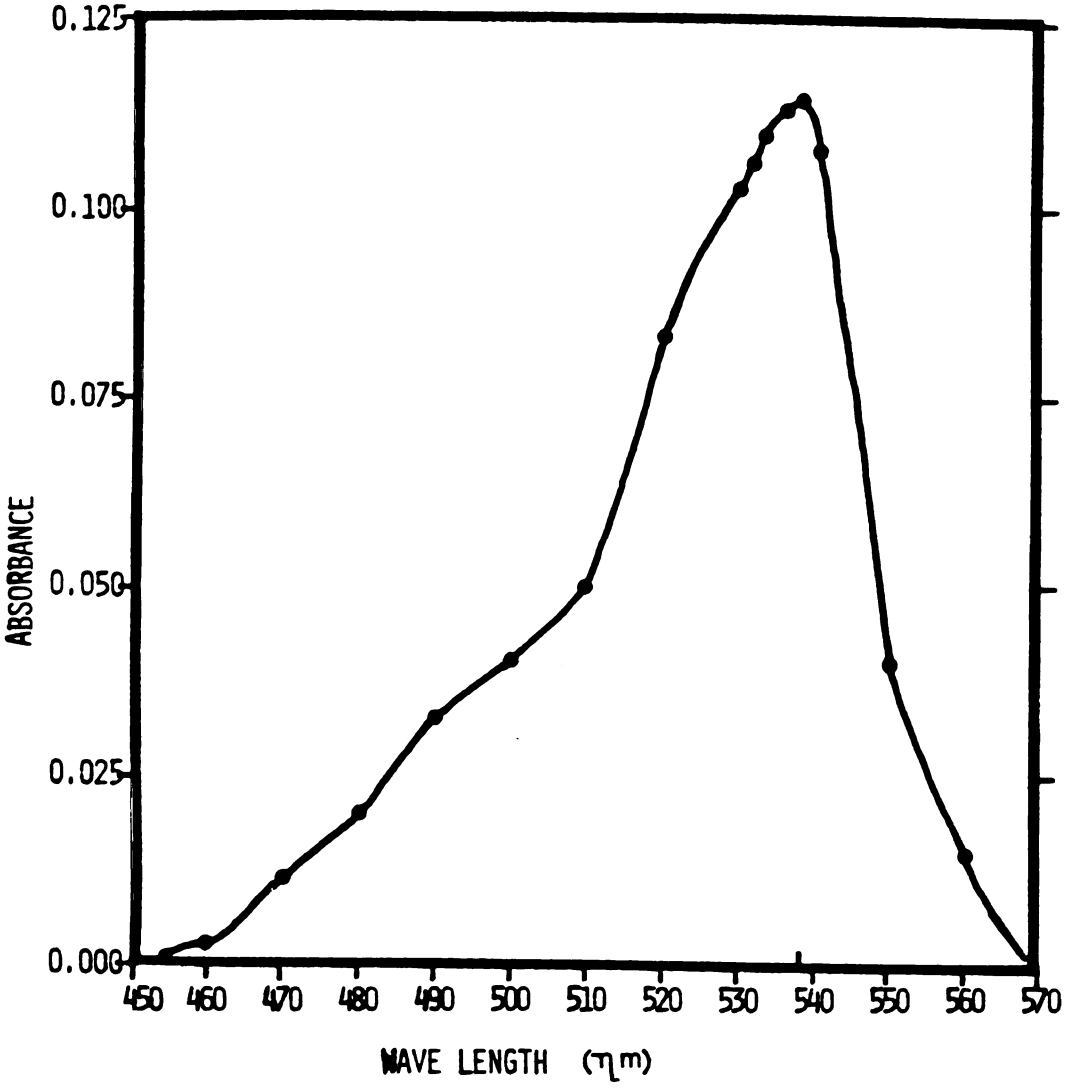


Figure 11. Absorption spectrum from malonaldehyde and 2-Thiobarbituric acid (TBA) complex from minced carp tissue.

factor K for converting absorbance values to TBA numbers, was conducted according to Tarladgis et al. (1960). A recovery of 68.4 to 71.3 percent was achieved in accordance with results reported by Tarladgis et al. (1960). A conversion factor of 7.8 was used to express absorbance readings as TBA numbers throughout this study.

Effect of Antioxidants and Chelators on TBA Numbers of Mechanically Deboned Carp During Frozen Storage

TBA values were determined and used as numerical indicators to assess the degree of lipid oxidation in mechanically deboned minced carp subjected to various antioxidant treatments. Initial TBA number for mechanically deboned minced carp was 0.86±0.14. TBA numbers determined at various storage time intervals through eleven months of frozen storage ($18^{o}C$) for all the product treatments are listed in Table 9.

TBA numbers increased slightly during the first three months of frozen storage, increased more rapidly during the 4 to 8 month period and then increased rather slowly from 8 to 11 months of storage. This trend is somewhat similar to the general pattern of TBA values as reported by Deng et al. (1977) for changes occurring in frozen mullet fillets. A similar trend with time of frozen storage was reported by Morris and Dawson (1979) in sucker flesh (except that no leveling off trend was

Table 9. Mean TBA numbers[1] for mechanically deboned minced carp stored at -18°C for 11 months.

Treatment	Storage Time in Months						
	2	3	4	6	8	9	11
Control	0.97[a]	1.22[a]	2.83[a]	5.21[a]	6.95[a]	7.60[a]	8.10[a]
EDTA	0.87[a]	0.91[a]	1.89[b]	5.10[a]	5.53[a]	7.82[a]	8.21[a]
Citric Acid	0.93[a]	1.82[a]	2.12[a]	5.00[a]	6.89[a]	7.15[a]	8.09[a]
Ascorbic Acid	1.01[a]	1.00[a]	2.10[a]	4.92[a]	6.01[a]	6.70[a]	7.71[a]
Propyl Gallate	0.98[a]	0.88[a]	2.20[a]	4.48[a]	5.52[b]	6.80[a]	7.13[b]
BHA	1.02[a]	1.86[b]	1.92[b]	4.58[a]	5.40[b]	6.20[b]	6.89[b]
BHT	0.89[a]	1.90[b]	1.45[b]	4.30[a]	4.62[c]	6.50[b]	6.80[b]
BHA & BHT	1.13[a]	1.82[b]	1.74[b]	3.83[b]	5.00[b]	5.71[b]	6.35[b]
BHA + BHT + CA	1.20[a]	1.24[a]	1.80[b]	3.57[b]	4.52[c]	5.51[c]	4.97[c]
BHA + BHT + AA	1.12[a]	1.29[a]	1.41[b]	3.30[c]	4.19[c]	5.37[c]	6.01[c]
BHA + PG + CA	1.24[a]	1.66[a]	1.60[b]	3.21[c]	4.01[d]	4.93[c]	5.13[c]
BHA + PG + AA	1.13[a]	1.33[a]	1.71[b]	2.99[c]	3.55[d]	4.72[c]	5.32[c]
BHA + BHT + CA + PG	1.02[a]	1.39[a]	1.46[b]	2.10[d]	2.93[e]	3.91[d]	4.61[d]
BHA + BHT + AA + PG	1.00[a]	1.04[a]	1.34[b]	1.98[d]	2.54[e]	3.59[d]	4.31[d]
Freezgard®	1.05[a]	0.93[a]	1.62[b]	2.93[c]	4.08[d]	5.86[b]	6.13[c]
Tenox 2®	0.95[a]	0.95[a]	1.29[b]	1.88[d]	2.61[e]	4.00[c]	4.47[d]

[1]Mean of 2 replicates from 6 determination expressed as mg malonaldehyde/1000 g sample.

Note: Different letters in a column indicate significant difference ($p<0.05$) among treatments.

observed in later months of storage) and by Rippen (1981) in carp flesh.

TBA numbers indicated no significant differences among treatments during the first three months of storage, except for those products treated with BHA and BHT and the combination of BHA + BHT. These samples exhibited significantly higher TBA numbers in the third month of storage. No explanation could be attributed to this observation, except that it is known that phenolic antioxidants alone facilitate the release of free fatty acids (Mai and Kinsella, 1979), which might have influenced the result. The same phenolic antioxidants used in combination with chelators or propylgallate alone, did not result in significant differences in TBA numbers.

The TBA numbers for control samples doubled between the third month and the fourth month of storage while the TBA numbers of products receiving other treatments increased only slightly. The EDTA treated products had significantly lower TBA numbers than the control products. TBA values for all products except those treated with citric acid, ascorbic acid, and propylgallate were significantly lower than the untreated controls. TBA numbers for carp products after 6 months of storage showed that BHA, BHT and PG treated samples exhibited lower absolute TBA numbers, however they were not significantly lower than those of samples treated with

EDTA, citric acid, ascorbic acid, and the control itself. Samples treated with BHA + BHT and BHA + BHT + citric acid showed significantly lower TBA numbers than the earlier-mentioned samples, but were significantly higher than those of BHA + BHT + ascorbic acid, BHA + BHT + PG, BHA + PG + ascorbic acid and Freezgard®-treated samples. The lowest TBA numbers (significantly) at the end of 6 months of storage were found in the samples treated with BHA + BHT + citric acid + PG, BHA + BHT + ascorbic acid + PG and Tenox-2® treated samples.

After 8 months of storage, based on significant differences in TBA numbers, all the treatments could be grouped into four categories. The highest TBA values were recorded for the control, EDTA, citric acid, ascorbic acid, PG, BHA and BHA + BHT treated samples. These were followed by BHT, BHA + BHT + citric acid and BHA + BHT + ascorbic acid treated samples. Second lowest TBA numbers were observed for products with BHA + PG + CA, BHA + PG + ascorbic acid and Freezgard®treated samples and the lowest TBA numbers (least oxidation) were recorded for products treated with BHA + BHT + PG + citric acid, BHA + BHT + PG + ascorbic acid and Tenox 2® . It has been generally accepted that a combination of BHA + BHT in a 1:1 proportion gives good synergism and good protection against lipid oxidation (Bentz et al., 1952; Dugan, 1960; Uri, 1961; Stuckey, 1962; Sherwin, 1972).

Mechanically deboned minced carp treated with BHT alone had lower TBA values at the end of 8 months of frozen storage than did those treated with a BHA + BHT combination.

Products held for the 9 months of storage showed high TBA numbers with significantly lower TBA numbers observed for BHA + BHT + PG + ascorbic acid, BHA + BHT + PG + citric acid and Tenox 2® treated samples. At the end of eleven months of storage TBA numbers had increased slightly showing a plateauing trend. Lowest TBA numbers at 11 month storage were found in the same products which had lowest numbers after 8 and 9 months storage.

The effect of various antioxidant treatments on rancid odor and flavor of fish meat loaf as evaluated by a panel is shown in Figure 12. The panel scores were based on a hedonic scale of 0 = very fresh and no rancid flavor or odor and 5 = undesirable rancid odor and flavor. General agreement was found between TBA numbers and rancid flavor and odor scores was observed (correlation coefficient - 0.87). Although large standard deviation and standard errors were found, the scoring trend was inversely related to the TBA numbers. Arbitrary panel scores of 2.5 or below were selected to indicate acceptance and based on the TBA numbers of 2.6 to 3.5 was chosen to be reasonably well-preserved (acceptable) mechanically deboned frozen carp flesh. With

Figure 12.--Mean rancid odor and flavor scores for fish meat loaf prepared from mechanically deboned minced frozen carp stored up to 11 months.

this subjective limit, Tenox 2®, BHA + BHT + PG + ascorbic acid and BHA + BHT + ascorbic acid formulations were effective in preservation of mechanically deboned minced carp which was frozen and stored up to 8 or 9 months. The high correlation coefficient between TBA numbers and panel scores obtained in this study is similar to that reported by Deng et al. (1977) who showed a very high correlation coefficient (γ= -0.96) in frozen mullet. Earlier reports, however, showed a poor relationship between actual TBA numbers and panel scores for rancid odors. The malonaldehyde measured does not in itself make a significant contribution to the oxidized odors and flavors. Since malonaldehyde forms one of the main intermediary compounds in the breakdown process of fatty acids leading to lipid oxidation, and at any given time this is a continuous process, the further breakdown of products of the malonaldehyde reaction results in the objectionable rancid odor and flavor. The usefulness of TBA numbers, even when a poor correlation exists between the analytical results and panel score, has been discussed by various authors (Keller and Kinsella, 1973; Jacobson and Koehler, 1970). The results obtained from this part of the study did show significant correlations between subjective and objective measurements of rancidity. As reported earlier, the lowest TBA numbers were achieved with Tenox 2®, BHA + BHT + PG +

ascorbic acid and BHA + BHT + ascorbic acid. Since Tenox 2® is commercially formulated, USDA approved and marketed, it could be an excellent source of antioxidants for storage of carp. It was surprising to note that although Tenox 2® is a combination of BHA + PG + citric acid, a laboratory combination of the same products did not result in low TBA numbers. Since PG can lead to color problems and ascorbic acid may increase water holding capacity, if one plans to separate white carp meat from dark meat for frozen storage, BHA + BHT + ascorbic acid combination may prove to be a useful antioxidant for this purpose. BHA + BHT + ascorbic acid has residual properties which are very useful, especially when mechanically deboned flesh is to be incorporated into a product that is to be deep fat fried.

Different antioxidant systems vary in their effectiveness when used in various combinations in different species of fish during frozen storage. Teeny and Miyauchi (1972) reported BHA, BHT, and their combinations to be effective in minimizing lipid oxidation in trout. Propyl gallate has been shown to be an effective oxidation inhibitor for bull fish. Deng et al. (1977) showed that TBHQ was an effective antioxidant for preserving mullet, while Morris and Dawson (1979) reported Freezgard to be very effective for sucker. In this study Tenox 2® BHA + BHT + ascorbic acid and BHA + BHT + PG + ascorbic

acid were found to be effective for preserving carp in long-term frozen storage. Rippen (1981) reported that Tenox 2® was an effective antioxidant mixture for carp during frozen storage.

EDTA in carp flesh resulted in significantly lower TBA numbers than control, ascorbic acid or citric acid treated samples during 4 months of storage. This may indicate that nonheme metal ions can play a role in increased TBA number formation and that the amount of ascorbic acid or citric acid used may not be sufficient to chelate all nonheme metal ions, which can influence lipid oxidation. Sodium EDTA was shown to be an effective antioxidant treatment for frozen storage of mullet (Deng et al., 1977). Sodium EDTA was also shown to effectively reduce rancidity in Spanish Mackeral (Farragut, 1972).

An explanation for some sudden decreases in TBA numbers during storage could be that lipid hydrolysis resulted in formation of free fatty acids and can be high enough to depress the oxidation of lipids (Castell et al., 1966). Degradation products of proteins and amino acids can interact with malonaldehyde and other lipid breakdown aldehydes (Kwon et al., 1965) and may be responsible for this phenomenon.

Awad et al. (1969) and Castell et al. (1966) suggested that the reaction of free fatty acids with

protein and their degraded components can form a protection against double bonds of fatty acids and may alter the oxidation process. Unavailability of malonaldehyde by various cross reactions is suggested as another reason for the variations found in TBA numbers during frozen storage (Buttkus, 1967). Lundberg (1962a) reported that malonaldehyde reacts with ε-amino groups of trout myosin. Variations in available oxygen affecting oxidation is given as an explanation for variations in TBA numbers during frozen storage (Lundberg, 1962a).

Ascorbic acid alone has been shown to be an effective antioxidant for frozen mullet (Deng et al., 1977) and for chub and cisco fillets (Greig, 1967). In this study ascorbic acid with various antioxidant combinations showed equal or better results compared to use of citric acid alone in controlling TBA numbers.

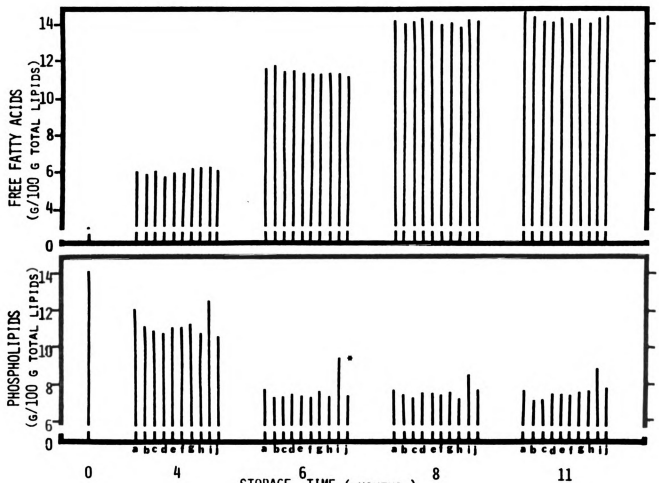
Effect of Storage Time and Treatments on Various Lipid Classes

Mechanically deboned minced carp and nine of the samples treated with various antioxidants were analyzed by TLC procedures for lipid classification following 0, 4, 6, 8, and 11 months of frozen storage. The lipid fractions separated were phospholipids, free fatty acids, triglycerides, mono and diglycerides, total cholesterol, and other unidentified fractions. These results are

shown in Figures 13, 14, and 15.

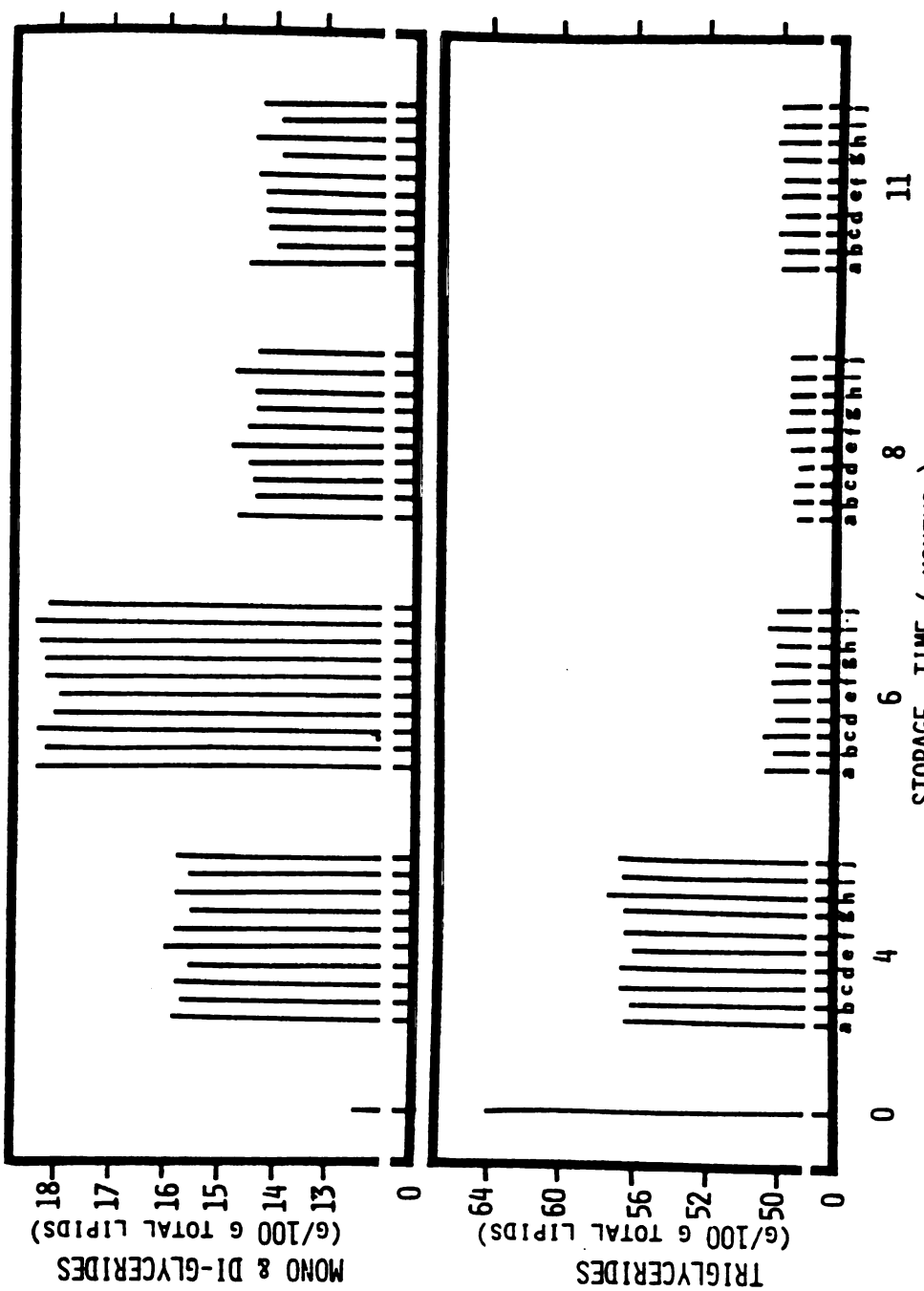
Irrespective of treatment, the total phospholipids expressed as a percentage of total lipids for products during those storage periods showed a significant decrease from 0 to 6 months of storage. Results from products held longer than 6 months showed no significant changes (Figure 13). Comparison among treatments for a given storage period showed that even though there were variations in percent total phospholipid, differences were not significant, except that Freezgard® treated sample had retained significantly more total phospholipids than the other samples at 6 months of frozen storage. After eleven months of storage, about 45 percent of the total phospholipids remained, of which about 20 percent loss occurred between the 4th and 6th month of storage. Although no significant differences were found, it was observed that phenolic antioxidants in presence of chelators had a tendency to retain a higher percent of the phospholipids.

Significant differences in free fatty acids were observed during the frozen storage of mechanically deboned minced carp. The free fatty acid content of 2.7 percent in carp increased to about 7.2 percent at the end of 4 months of storage, and increased to 11.6, 14.4 and 14.75 percent by the end of the 6th, 8th, and 11th months of storage, respectively (Figure 13).



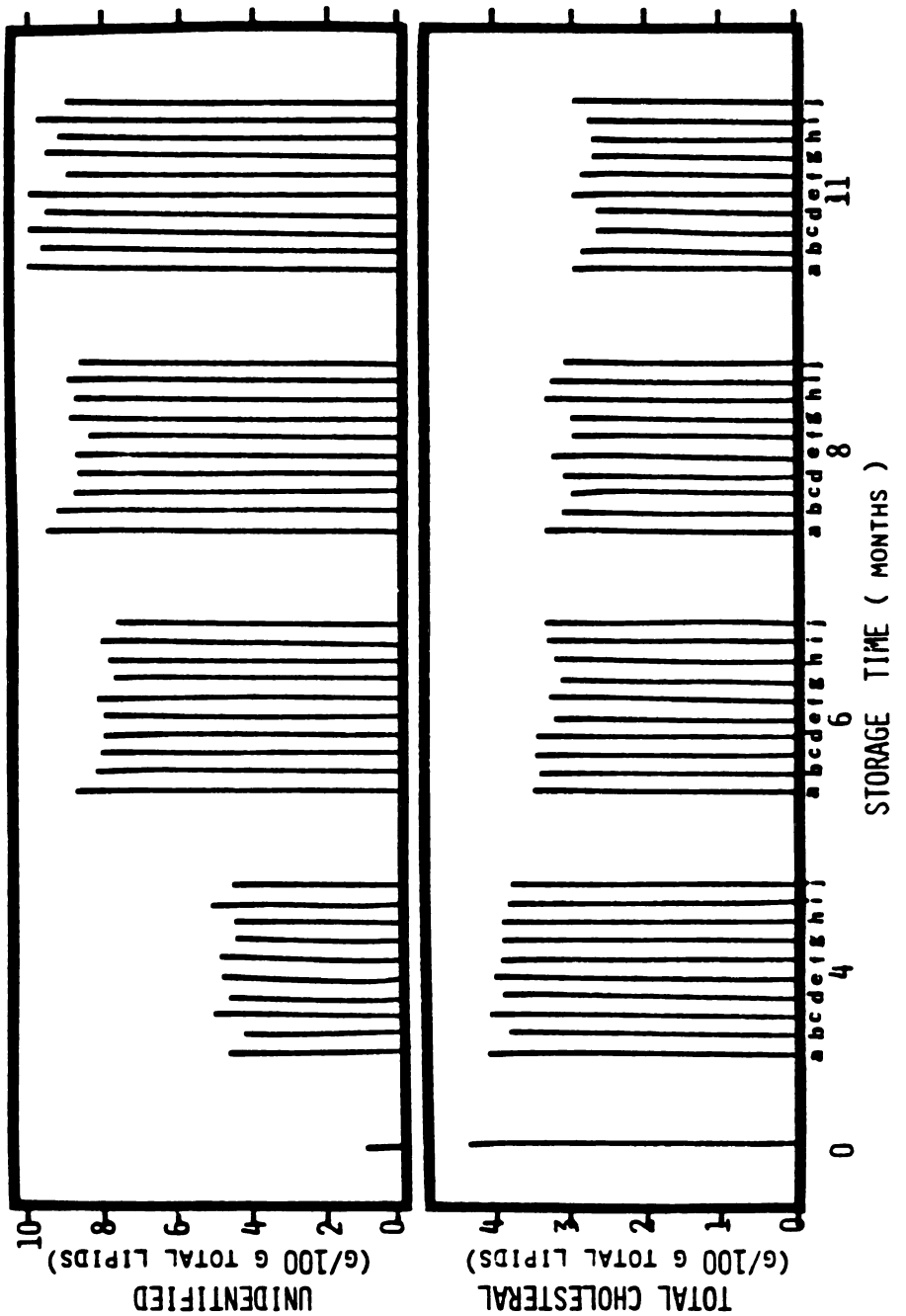
a = control, b - BHA + BHT, c = BHA + BHT + CA, d = BHA + BHT + AA, e = BHA + PG + CA
f = BHA + PG + AA, g = BHA + BHT + PG + CA, h = BHA + BHT + PG + AA, i = Freezgard®, j = Tennox 2®

Figure 13.--Effect of treatment and time on total phospholipids and free fatty acids in mechanically deboned minced carp during frozen storage



a = control, b = BHA + BHT, c = BHA + BHT + CA, d = BHA + BHT + AA. e = BHA + PG + AA, f = BHA + PG + AA, g = BHA + BHT + PG + CA, h = BHA + BHT + PG + AA, i = Freezgard®, j = Tennox 2®

Figure 14. Effect of treatment and time on triglycerides, and mono and diglycerides in mechanically deboned minced carp during frozen storage.



a = control, b = BHA + BHT, c = BHA + BHT + CA, d = BHA + PHT + CA, e = BHA + PG + CA, f = BHA + PG + AA, g = BHA + BHT + PG + CA, h = BHA + PHT + PG + AA, i = Freezgard®, j = Tennox 2®

Figure 15.--Effect of treatment and time on total cholesterol and "other" unidentifiable fractions in mechanically deboned minced carp during frozen storage.

No significant differences in free fatty acids among treatments at any given period of storage were found.

In initial control samples. 64.4 percent of total lipids were triglycerides. A loss of 7.86 and 16.9 percent occurred by the end of 4 and 6 months of storage, respectively. After 8 months of storage the triglyceride content decreased to about 77.2 percent of its original level (Figure 14). This remained the same through eleven months of storage. As observed in other fractions, no significant identifiable differences in the triglyceride fraction due to various treatments were found.

Figure 14 shows the change in total mono- and diglyceride contents of mechanically deboned minced carp treated with various antioxidants during eleven months of frozen storage. No significant differences were found in these chemical compounds due to treatment during any period of frozen storage.

Changes in total cholesterol contents and unidentifiable TLC spot fractions are presented in Figure 15. The total cholesterol content of control samples expressed as percentage (based on total lipids) decreased from 4.4 to 4.2, 3.9, 3.7, 3.7, and 3.7 percent, respectively, at the end of 0, 4, 6, 8, and 11 months of frozen storage. At any given storage time, the values obtained from samples from each treatment did not differ significantly. A 1 percent unidentifiable fraction at 0 month increased

to about 4.6 percent and 8.8 percent, respectively, by the end of 4 and 6 months of storage and then leveled off at about 10 percent. No significant differences in total cholesterol due to treatment were observed.

Loss of phospholipids and formation of free fatty acids have been reported for stored fish muscle (Dyer and Fraser, 1959). Presumably lipid hydrolysis in foods is an enzymic reaction (Lea, 1962) involving phospholipids, the triglycerides, and other lipid constituents.

During frozen storage, a decrease in the content of phospholipids in chicken muscle and an increase in free fatty acids, and triglycerides were reported by Davidkova and Khan (1967). In this study, no increase in the triglyceride fraction was found; however, the mono and diglyceride fractions did increase during frozen storage. Following a decrease in lipid fractions and their comparison with increasing free fatty acids in fish muscle, the lipid degradation was attributed to both hydrolytic and oxidative reactions during frozen storage (Takama et al., 1967). Takama et al. (1967) revealed a 61 percent decrease in the total triglyceride fraction at the end of 100 days storage for blue fin tuna stored at -20°C. Triglyceride hydrolysis of Atlantic herring during frozen storage was also reported by Olley and Lovern (1960). Similar results were reported by Bosund and Ganrot (1969a).

Effect of Storage Time and Treatments on Various Phospholipid Fractions

Six different phospholipid fractions, phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomylin (SM), phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (C) were separated from carp samples from each treatment at the end of 4, 6, 8, and 11 months. When the results were expressed as mg phospholipid/100 g tissue for any given storage time, no significant differences were observed among treatments. Changes in phospholipid fractions from carp muscle from various treatments are represented in Figure 16. Generally, the phospholipid fractions decreased during the first six months of storage and changed little thereafter. Phosphatidylcholine and phosphatidylethanolamine, which accounted for more than 50 percent of total phospholipids, showed the most degradation of all the fractions. PC decreased by 24 percent and 56 percent by the end of 4 and 6 months, respectively, while PE decreased by 30 percent and 61 percent during the same storage period. After 6 months, SM, PS, PI, and C decreased by about 37, 19, 29 and 39 percent, respectively. From 6 months through 11 months of storage, no appreciable variation in any phospholipid fraction was observed. However, it was noticed that most of the fractions showed a slight increase during this period. The sum of all unidentifiable fractions amounted

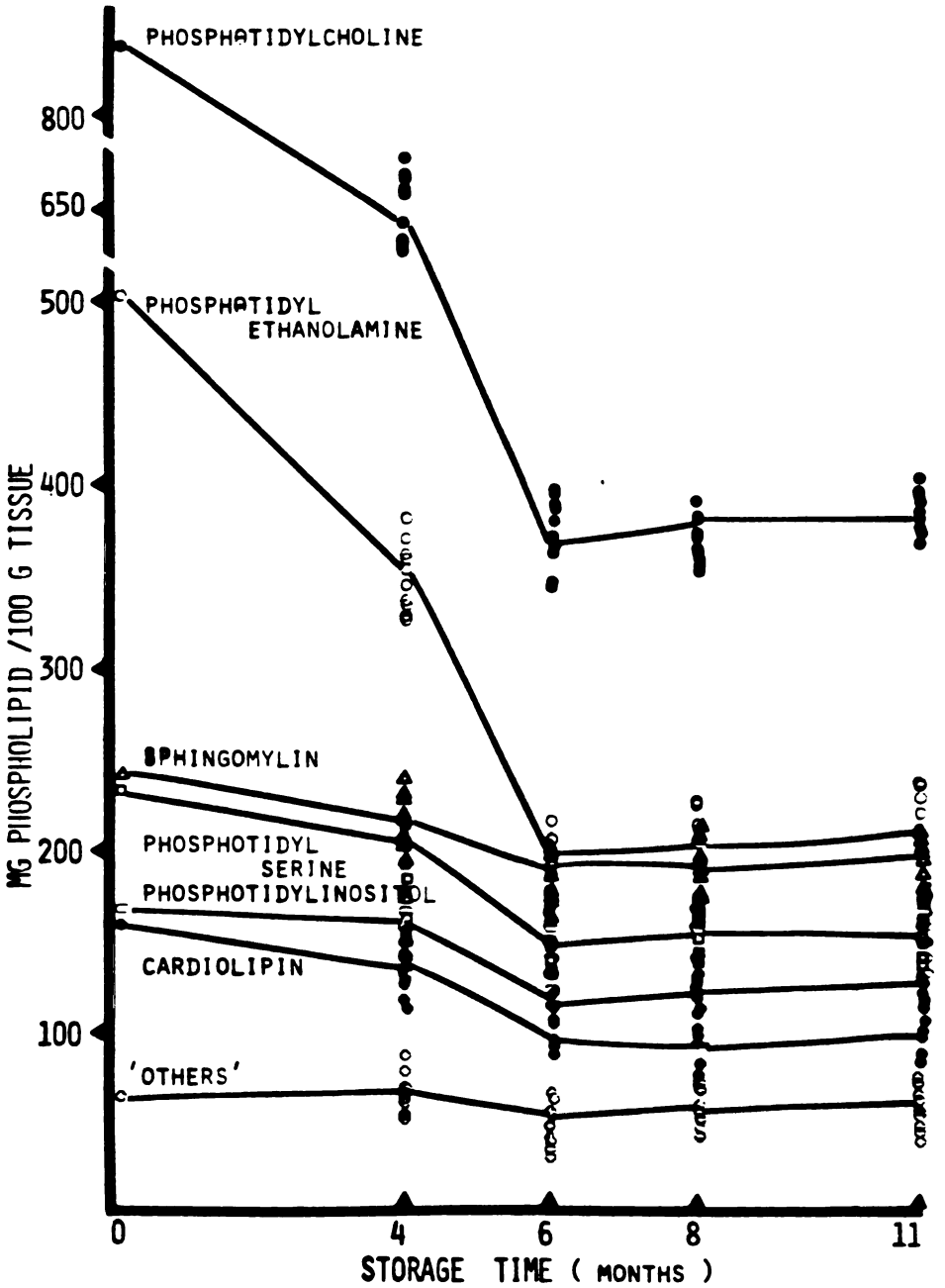


Figure 16.--Effect of antioxidant treatments and time on various phospholipid fractions in mechanically deboned minced carp during 11 months of frozen storage.

to about the same throughout the storage period. Various changes in the phospholipid fractions were most pronounced during the 4th and 6th month of storage and then leveled off. Loss of phosphatidylcholine and phosphatidylethanolamine accounted for most of the decreases. Numerous studies have shown lipolysis of phospholipids during frozen storage in bovine, fish, and chicken muscle (Awad et al., 1969; Bosund and Ganrot, 1969b; Davidkova and Khan, 1967; Takama et al., 1967). Enzymatic hydrolysis may play a predominent role. For example, it is known that mammalian tissues containing phospholipases continuously release fatty acids from phosphoglycerides (McMurray and Magee, 1972). The rapid decrease in phosphatidylcholine and phosphatidylethanolamine (and the remaining fractions) in the first half of storage period followed by leveling off may be due to the increased surface area of the mechanically deboned carp fascilitating oxidation (Castell et al., 1970; Lea, 1957). Rancidity in meat during frozen storage has been related to breakdown of the phospholipids (Caldwell et al., 1960; Greene, 1971; Lea, 1957; Love and Pearson, 1971; Keller and Kinsella, 1973).

Effect of Storage Time and Treatments on Fatty Acid Fractions from Phospholipids

Total saturated, monoenic, and polyenoic fatty acid fractions from carp muscle subjected to various treatments

at the end of 4, 6, 8, and 11 months of frozen storage are listed as mg fatty acid/100 g of tissue (Table 10). Variations in the ratios of total saturated fatty acids to total polyenoic acids are also listed in Table 10. In general, saturated fatty acids followed by monoenoic fatty acids declined less than did polyenoic acids. For example, a loss of about 20 percent of saturates and 32 percent of monoenoic fatty acids occurred in control samples, while a loss of 66 percent of polyenoic acids was observed during eleven months of frozen storage. This difference is highly significant, since polyenes form nearly 50 percent of the phospholipid fatty acids in mechanically deboned minced carp. The fatty acids 18:3, 20:4, 20:5 ω 6 and 22:6 acids totaled about 80 percent of the total polyenoic acids from phospholipids. No significant differences in unsaturation ratios occurred among the samples due to treatments during the 4th month of frozen storage. At the end of 6 months of storage, samples treated with BHA + BHT + PA + CA, BHA + BHT + PG + AA and Tenox 2® had significantly higher saturation ratios than did samples receiving other treatments. This accounted for an 80 to 85 percent retention of polyenes for these treatments compared to 45 to 70 percent for the rest of the treatments. These treatments also showed significantly higher retention of polyenes at the end of 8 months of storage. However, by the end of 11 months

Table 10. Effect of time and antioxidant treatment on the phospholipid fatty acid fractions and ratio of saturate to unsaturated fatty acids in mechanically deboned carp during frozen storage.

Treatment F	Storage Time (months)							
	4		6		8		11	
	FA	R	FA	R	FA	R	FA	R
Control	S 567		532		515		490	
	M 325	1.98[a]	308	1.39[a]	290	1.29[a]	255	1.19[a]
	P 796		431		374		328	
BHA+BHT	S 604		567		541		530	
	M 343	1.96[a]	325	1.54[a]	312	1.42[a]	276	1.30[a]
	P 841		548		455		413	
BHA+BHT+CA	S 583		547		531		540	
	M 333	2.06[a]	328	1.58[a]	309	1.47[a]	278	1.40[a]
	P 824		537		471		478	
BHA+BHT+AA	S 571		536		519		516	
	M 333	2.03[a]	316	1.77[a]	300	1.58[a]	269	1.51[a]
	P 824		632		519		509	
BHA+PG+CA	S 593		554		520		512	
	M 356	1.99[a]	338	1.72[a]	321	1.57[a]	296	1.53[a]
	P 827		617		495		489	
BHA+PG+AA	S 619		598		544		534	
	M 359	2.00[a]	341	1.77[a]	324	1.62[a]	298	1.59[a]
	P 879		687		560		550	
BHA+BHT+PG+CA	S 619		575		536		540	
	M 373	2.08[a]	356	1.96[b]	341	1.80[b]	298	1.74[b]
	P 893		771		659		641	
BHA+BHT+PG+AA	S 639		600		582		570	
	M 370	2.00[a]	350	1.99[b]	337	1.86[b]	321	1.78[b]
	P 907		844		745		693	
Freezgard®	S 594		555		527		523	
	M 361	2.10[a]	342	1.75[a]	311	1.60[a]	287	1.15[b]
	P 885		629		548		502	
Tenox 2®	S 619		582		564		547	
	M 377	2.09[a]	359	1.95[b]	340	1.79[b]	328	1.76[b]

Note: Different superscripts in the same column represent significant differences at $p<0.05$.

FA = Fatty acid fraction; FA = Mg fatty acid/100 g tissue;

R = Ratio of USFA/SFA; S = Total saturated fatty acids;

M = Total monoenez; P = Total polyenes

of frozen storage, the control , BHA + BHT and BHA + BHT + CA treated samples showed only 33 to 49 percent retention of polyenoic acid while other treatments showed 53 to 65 percent retention of polyenes.

It is known from earlier reports that neutral lipid fractions in various kinds of meat undergo oxidation more slowly than phospholipids (Awad et al., 1969; Bosund and Ganrot, 1969b; Davidkova and Shan, 1967; Takama et al., 1967).

During frozen storage of mackerel and blenny, Takama (1974) reported a decrease in C20:5, C22:6, and C18:1 fatty acids and in lecithin during frozen storage. Similar results were reported for herring by Bosund and Ganrot (1969b). Oxidation of neutral and phospholipids has been expressed in terms of a decrease in C22:6 fatty acid during storage of Jack Mackerel (Shono and Toyomizu, 1971, 1973). In this study, content of lipids from control carp flesh lipid declined 59 percent in the C22:6 acid during 11 months of storage. Also C18:3, C20:1, C20:4, and C20:5 ω 6 acids decreased substantially. The ratio of saturated fatty acids to unsaturated fatty acids from phospholipids decreased from 1.98 to 1.19 during frozen storage. Most of the literature reviewed reported various effects of antioxidants on lipid fractions such as phospholipids and neutral lipids rather than specific fatty acids. Mai and Kinsella (1979)

reported no significant changes in fatty acid composition during 8 weeks of frozen storage of mechanically deboned carp, and BHA and TBHQ did not result in superior retention of fatty acids. This was expected since hydrolysis of lipids occurs rather slowly during frozen storage and oxidation of fatty acids would take time after accumulation of free fatty acids and the only variation in fatty acid retention would occur depending on the effectiveness of the antioxidant used. In this study a higher retention of unsaturated phospholipids occurred in products treated with BHA + BHT + PG, in presence of citric acid or ascorbic acid and Tenox 2® at the end of 8 months of frozen storage. These observations relate to the significantly higher ratio of saturated to unsaturated fatty acids (Table 10).

Effect of Storage Time and Treatments on Extractability of Sarcoplasmic and Myofibrillar Proteins

Water soluble and salt soluble protein nitrogen was designated as sarcoplasmic and myofibrillar proteins. Values of protein nitrogen of these two fractions from mechanically deboned minced carp treated with various antioxidants are listed in Table 11. Both fractions decreased during storage. Sarcoplasmic proteins decreased by about 4 to 18, 14 to 38, and 32 to 42 percent at the end of 3, 8, and 11 months storage, respectively.

Table 11. Effect of time and antioxidant treatment on extractability of sarcoplasmic and myofibrillar protein nitrogen from mechanically deboned minced carp during frozen storage.

Antioxidant Treatment	Storage Time (months)					
	3		8		11	
	SP[1]	MF[2]	SP	MF	SP	MF
Control	325[a]	422[a]	260[a]	372[a]	232[a]	304[a]
BHA+BHT	327[a]	436[a]	264[a]	389[a]	227[a]	321[a]
BHA+BHT+CA	378[b]	421[a]	296[a]	380[a]	248[a]	327[a]
BHA+BHT+AA	364[a]	429[a]	298[a]	374[a]	247[a]	314[a]
BHA+PG+CA	332[a]	434[a]	270[a]	377[a]	216[a]	321[a]
BHA+PG+AA	351[a]	431[a]	252[a]	368[a]	217[a]	313[a]
PHA+BHT+PG+CA	360[a]	455[b]	337[b]	421[b]	267[a]	346[b]
BHA+BHT+PG+AA	349[a]	445[a]	328[b]	426[b]	254[a]	361[b]
Freezgard®	338[a]	439[a]	321[b]	411[a]	257[a]	348[b]
Tenox 2®	381[b]	462[b]	342[b]	419[b]	259[a]	349[b]

Note: Values are mean of 2 determinations.

[1]SP = mg sarcoplasmic nitrogen/100 g tissue.

[2]MF = mg myofibrillar nitrogen/100 g tissue.

[3]Different superscripts in a column represent significant differences at $p<0.05$.

Myofibrillar protein fractions changed from 6 to 14, 13 to 24, and 26 to 38 percent after 3, 8 and 11 months, respectively. Statistically, samples containing BHA + BHT + CA and Tenox 2® retained a higher percent of soluble sarcoplasmic protein than other samples after three months of storage. After 8 months of storage, samples treated with BHA + BHT + PG + CA, BHA + BHT + PG + AA, Freezgard® and Tenox 2® had significantly higher retentions of soluble sarcoplasmic proteins, and the same treatemtns (except for Freezgard®) resulted in similar retentions of myofibrillar proteins. After eleven months storage, extractability of sarcoplasmic proteins did not differ significantly among various treatments. The solubility of myofibrillar proteins from BHA + BHT + PG + AA, BHA + BHT + AA, Freezgard® and Tenox 2® treated samples was higher than for the remainder of samples.

Effect of Storage Time and Treatments on the Shear Values of Fish Gels

Mean shear values (kg force/g sample) of fish gels subjected to various treatments and stored frozen for 3, 8 and 11 months are listed in Table 12. Initial shear value was referred to as 100 percent and shear values decreased with storage time. This decrease was equal to about 13 percent (some samples showing slight increase), 16 to 42 percent, and 28 to 57 percent after 3, 8, and

Table 12. Effect of time and antioxidant treatment on shear values of fish gels prepared from mechanically deboned minced carp during frozen storage.

Antioxidant Treatment	Storage Time (months)		
	3	8	11
Control	1.43[a]	1.08[a]	0.80[a]
BHA + BHT	1.59[a]	1.19[a]	0.91[a]
BHA + BHT + CA	1.70[a]	1.39[a]	0.98[a]
BHA + BHT + AA	1.73[a]	1.47[a]	1.01[a]
BHA + PG + CA	1.62[a]	1.58[b]	0.93[a]
BHA + PG + AA	1.91[b]	1.50[b]	0.86[a]
BHA + BHT + PG + CA	1.90[b]	1.40[a]	1.11[a]
BHA + BHT + PG + AA	1.97[b]	1.56[b]	1.17[a]
Freezgard®	2.07[b]	1.59[b]	1.37[b]
Tenox 2®	1.93[b]	1.46[a]	1.09[a]

Note: Values are mean of 2 determinations.

Kg force/g sample.

Different superscripts in a column represent significant difference, $p<0.05$.

11 months of storage, respectively, for various treatments. During the initial period of analysis, significantly lower shear values were found for samples containing BHA + PG + AA, BHA + BHT + PG + CA, BHA + BHT + PG + AA, Freezgard® and Tenox 2®. After 8 months of storage the shear values for samples treated with BHA + BHT + AA, BHA + PG + CA, BHA + PG + AA, BHA + BHT + PG + AA and Freezgard® had signficantly higher shear values than those from other treatments. No significant differences in shear values were found among the treatments at 11 months of storage.

Effect of Storage Time and Antioxidant Treatments on Waterholding Capacity

Fish gels were centrifuged and the percent extractable liquids were expressed as waterholding capacity. Mean percent of liquids extracted are listed in Table 13. The extractable liquids increased during storage for all treatments. The values for products stored 3, 8, and 11 months increased from 1 to 12 percent, 4 to 22 percent, and 12 to 34 percent, respectively. At the end of three months, BHA + BHT + AA, BHA + PG + AA, BHA + BHT + PG + AA and Freezgard® treated samples showed significantly lower extractable liquids (higher waterholding capacity) than the rest of the samples. After eleven months of storage (except for control), BHA + BHT, BHA + BHT + CA and Tenox 2® treated samples had significantly higher waterholding capacities (or lower percent of extractable liquids) than the rest of

Table 13. Effect of time and antioxidant treatment on water holding capacity (percent liquid extractable) of fish gels prepared from mechanically deboned minced carp during frozen storage.

Antioxidant Treatment	Storage Time (months)		
	3	8	11
Control	19.2[1]	21.1[a]	24.0[a]
BHA + BHT	18.8[a]	20.4[a]	22.2[a]
BHA + BHT + CA	18.3[a]	20.2[a]	21.6[a]
BHA + BHT + AA	18.0[b]	18.2[b]	19.7[b]
BHA + PG + CA	18.3[a]	20.0[a]	21.3[b]
BHA + PG + AA	17.9[b]	18.2[b]	19.2[b]
BHA + BHT + PG + CA	18.1[a]	20.3[a]	20.9[b]
BHA + BHT + PG + AA	18.0[a]	17.9[b]	19.2[b]
Freezgard®	17.8[b]	18.3[b]	19.5[b]
Tenox 2®	18.5[a]	19.1[a]	21.0[a]

Note: Values are mean of 2 determinations.

[1]Different superscripts in a column represent significant difference at $p<0.05$.

the antioxidant treated samples.

In red meat, salt soluble proteins (myofibrillar proteins) are responsible for desired textural properties of muscle protein emulsion type comminuted products (Fukazawa et al., 1961; Hegarty et al., 1963; Samejima et al., 1969; Trautman, 1970; Swift, 1965; Tasi et al., 1972; Nakayama and Sato, 1971). The results in this study indicate that both the sarcoplasmic and myofibrillar protein fractions from carp decreased during frozen storage (Table 11). Similar results were reported by Cheng et al. (1979a), however, the decrease in sarcoplasmic protein fractions noted in this study were more noticeable than they reported. No particular reason could be attributed to this observation other than the variation due to species difference and comparatively slower method of freezing at lower temperatures (-26^{o}C) used in this study. Usually commercial plants freeze fish by plate freezers at -36^{o}C. The antioxidant treatments resulting in significantly lower TBA numbers did not necessarily lower the free fatty acid production, but did result in a higher protein extractability (γ =-0.40) and may suggest that the fatty acid degradative products may be more active in protein degradation rather than just free fatty acid formation itself during frozen storage of carp flesh. A firm texture (high shear values) and better waterholding capacity (less water extracted by centrifugation) are

correlated with a high soluble myofibrillar fraction (Lee and Toledo, 1976; Cheng et al., 1979). The results in this study indicate that a similar relationship existed between waterholding capacity, shear values, and fractional solubilization of protein, specially for product treatments which resulted in low TBA values. No significant correlations between these factors, specially waterholding capacity and shear values, were identified.

Effect of Packaging Environment on Storage Stability of Mechanically Deboned Carp

Mechanically minced carp were packaged using atmospheric air, vacuum, and vacuum with nitrogen back flush. Frozen samples stored at -20°C up to eleven months were withdrawn at various intervals of time for further analyses. The specific purpose of this study was to evaluate the possible advantages of using nitrogen or vacuum for improving stability of mechanically deboned minced carp flesh during frozen storage.

The TBA numbers determined for products processed under various packaging treatments are listed in Table 14. TBA numbers from nitrogen and vacuum packed samples were slightly lower than those air packaged samples. However, an analysis of variance did not show significant differences between them. This may indicate that there is little advantage in using nitrogen or vacuum packaging for storing

Table 14. Effect of storage environment on TBA numbers* from mechanically deboned minced carp during frozen storage.

Storage Environment	Storage Period (monthly)						
	0	2	4	6	8	9	11
Air	0.97	1.22	2.83	5.21	6.95	7.60	8.10
Vacuum	0.93	1.30	2.41	5.09	6.70	7.51	7.86
Nitrogen	0.94	1.02	2.30	5.05	6.52	7.50	7.80

*Mg malonaldehyde/100 g sample.

Values are mean of 2 replicates with 6 determinations.

mechanically deboned minced carp flesh.

Jantawat (1978) reported similar results for mechanically deboned turkey meat held on ice for 72 hours prior to freezing, but for immediately frozen mechanically deboned turkey or chicken, she reported that vacuum and nitrogen packing significantly reduced TBA numbers. Most packaging environment studies have stressed the gas packaging effect on the storage stability from a microbiological point of view (Coyne, 1933; Killeffer, 1930; Shewan, 1950). The advantages of varying frozen storage environments of carp flesh storage, based on data from this study appear to be limited. However, it has been observed in fried and roasted foods that the packaging environments do reduce TBA numbers and may increase shelf life by limiting lipid oxidation (King, 1981; Naidu and Dawson, 1983).

Effect of Packaging Environment on Fatty Acid Fractions of Phospholipids

Total saturated, monoenoic, and polyenoic fatty acid fractions from carp packaged in air, nitrogen, and vacuum are listed as mg fatty acid/100 g tissue (Table 15). The ratios of unsaturated fatty acids to those of saturated fatty acids are listed. By the end of 8 months of frozen storage, air and vacuum packaged samples retained 60.08 and 62.44 percent, respectively, of total phospholipid fatty acids as compared to 68.26 percent retention for

Table 15. Effect of storage environment on various groups of phospholipid fatty acids and ratio of unsaturated fatty acids to saturated fatty acids in mechanically deboned minced carp stored at -20°C for eleven months.

Storage Environment		Storage Period (months)									
		0		4		8		9		11	
		mg/100 g	UR	mg/100 g	UR	mg/100 g	UR	mg/100 g	UR	mg/100 g	UR
Air	Saturates	630		567		529		517		491	
	Monoenes	376	2.14	323	1.98	308	1.39	289	1.27	256	1.20
	Polyenes	973		798		438		370		331	
Nitrogen	Saturates	653		594		575		548		536	
	Monoenes	389	2.12	346	2.02	323	1.63*	319	1.43*	315	1.22
	Polyenes	994		855		616		467		338	
Vacuum	Saturates	624		580		537		524		517	
	Monoenes	367	2.12	324	1.90	305	1.38	297	1.31	286	1.21
	Polyenes	954		781		437		390		314	

UR = Ratio of USFA/SFA.

All values other than UR are mean of two replications and are expressed as mg fatty acid/100 g wet tissue.

* in a column represented significant difference at $p<0.05$.

those packaged in nitrogen. The higher retention was due to less degradation of polyenes, since the variation due to packaging treatment among saturates and monoenes was minimum. However, there were no statistically identifiable advantages due to packaging treatment at end of eleven months of storage. From 4 to 9 months of storage, nitrogen packaged samples had a higher ratio of unsaturated to saturated fatty acids, with the ratio at 8 and 9 months being significantly different from that of 4 months.

Using mechanically deboned chicken and turkey meat, Jantawat (1978) observed that vacuum and nitrogen packaging treatments resulted in significantly higher retention of polyenoic fatty acids during four months of storage. However, in this study it was observed that only the nitrogen package showed some advantage in improved retention of polyenoic fatty acids of carp tissue over a period of 8 to 9 months. A possible explanation of nitrogen back flush having advantages over vacuum packaging would be the fact that the limited oxygen left after vacuum packaging is further diluted by nitrogen addition and hence decreases availability of oxygen for lipid oxidation. A general trend shows a decrease in oxygen due to vacuum and/or N back flush resulted in unsaturated fatty acid retention and decreased TBA numbers.

The effect of storage environment and antioxidant treatments on the TBA numbers at the end of 4, 8, and 11

months of storage of mechanically deboned minced carp are listed in Table 16. No significant differences were found among samples packaged in air, vacuum, and nitrogen. The TBA numbers for samples packaged using a nitrogen back-flush were slightly lower than all others. This difference, among packaging treatments, became slight after eleven months of storage. The packages did retain the vacuum and nitrogen tensions at the end of eleven months, however, no effort was made to measure the exact amount of vacuum or nitrogen retained. Deng et al. (1977) reported that vacuum packaging in combination with antioxidants improved storage stability of mullet as indicated by TBA numbers and peroxide values. In this study vacuum packaging did not improve the antioxidant effect, while a nitrogen back flush in combination with antioxidants did result in low TBA numbers. This may be due to the differences in vacuum levels used (the vacuum levels used in above author's experiment are not reported) and the differences in type and quantity of lipids, between carp and mullet.

Frozen Storage Stability of Hand Filleted Carp

Carp fillets with and without skin treated with Tenox 2®, Freezgard®, BHA + BHT + PG + citric acid, and BHA + BH + PG + ascorbic acid were frozen and stored at -20°C to monitor rancidity development.

Table 16. Mean TBA numbers[1] for mechanically deboned minced carp treated with various antioxidants and stored in presence of various packaging environments at -18°C for 11 months.

Antioxidants	Storage Time (months)								
	4			8			11		
	A[2]	V	N	A	V	N	A	V	N
Control	2.83	2.41	2.30	6.95	6.70	6.52	8.10	7.86	7.80
BHA	1.92	1.64	1.54	5.40	5.32	5.26	6.89	6.81	6.70
BHT	1.45	1.27	1.22	4.62	4.50	4.28	6.80	6.80	6.65
BHA + BHT	1.74	1.46	1.38	5.00	4.89	4.69	6.35	6.23	6.18
BHA + BHT + Citric acid	1.80	1.51	1.45	4.52	4.39	4.28	5.97	5.86	5.70
BHA + BHT + Ascorbic acid	1.41	1.20	1.18	4.19	4.07	4.00	6.01	6.02	5.92
BHA + PG + Citric acid	1.60	1.32	1.28	4.01	3.96	3.79	5.63	5.49	5.34
BHA + PG + Ascorbic acid	1.71	1.40	1.34	3.55	3.47	3.36	5.32	5.27	5.19
BHA + BHT + PG + Citric acid	1.46	1.21	1.17	2.43	2.81	2.70	4.61	4.50	4.29
BHA BHT + PG + Ascorbic acid	1.34	1.18	1.14	2.54	2.40	2.32	4.31	4.37	4.24
Freezgard®	1.62	1.31	1.30	4.08	3.90	3.82	6.13	6.05	5.20
Tenox 2®	1.29	1.16	1.13	2.61	2.55	2.47	4.47	4.41	4.34

[1]Mean of 2 replications from 6 determinations expressed as mg Malonaldehyde/kg sample.

[2]Air = Air, V = Vacuum, N = Nitrogen back flush.

BHA analysis (Doeden et al., 1979) as an index of total antioxidant content of frozen fillets after one day of treatment indicated a content between 0.016 to 0.018 percent of total antioxidant combination present in fillets without skin while that in fillets with skins ranged from 0.013 to 0.016 percent. The later concentration may be the result of removing the skin in obtaining flesh for lipid extraction. No analyses were performed to find the concentration of Freezgard® since the ratio of its components was not revealed by the manufacturer.

Evaluation of Fillets

Samples from each group of fillets with and without skin were examined periodically for TBA numbers, fatty acid changes, protein solubility, WHC, shear values, and taste panel evaluations for rancidity.

Graphic representations of the mean TBA numbers determined for various antioxidant treated hand filleted carp with and without skin stored at -20°C for eleven months are shown in Figures 17 and 18. The TBA numbers for the control fillets with and without skin were slightly lower than for the controls from mechanically deboned minced carp throughout the storage period.

Between the controls from fillets with and without skin, the skinless fillets had slightly higher TBA numbers throughout the storage period, with significant

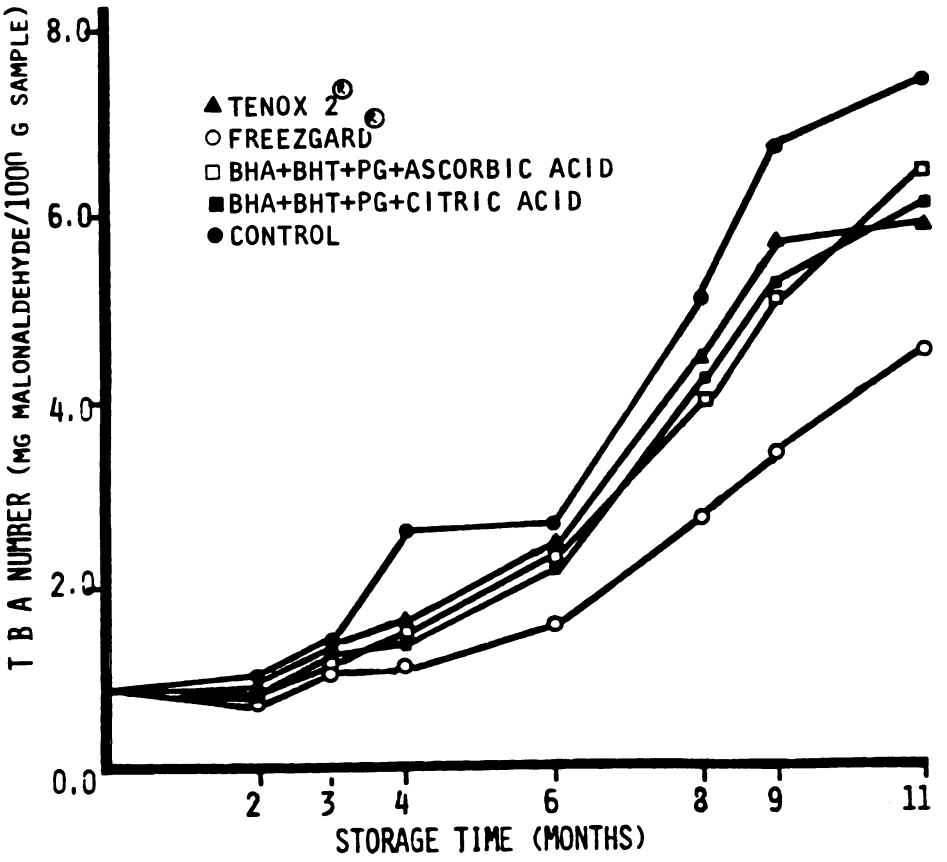


Figure 17.--Effect of antioxidants and frozen storage on TBA numbers of hand filleted carp with skin.

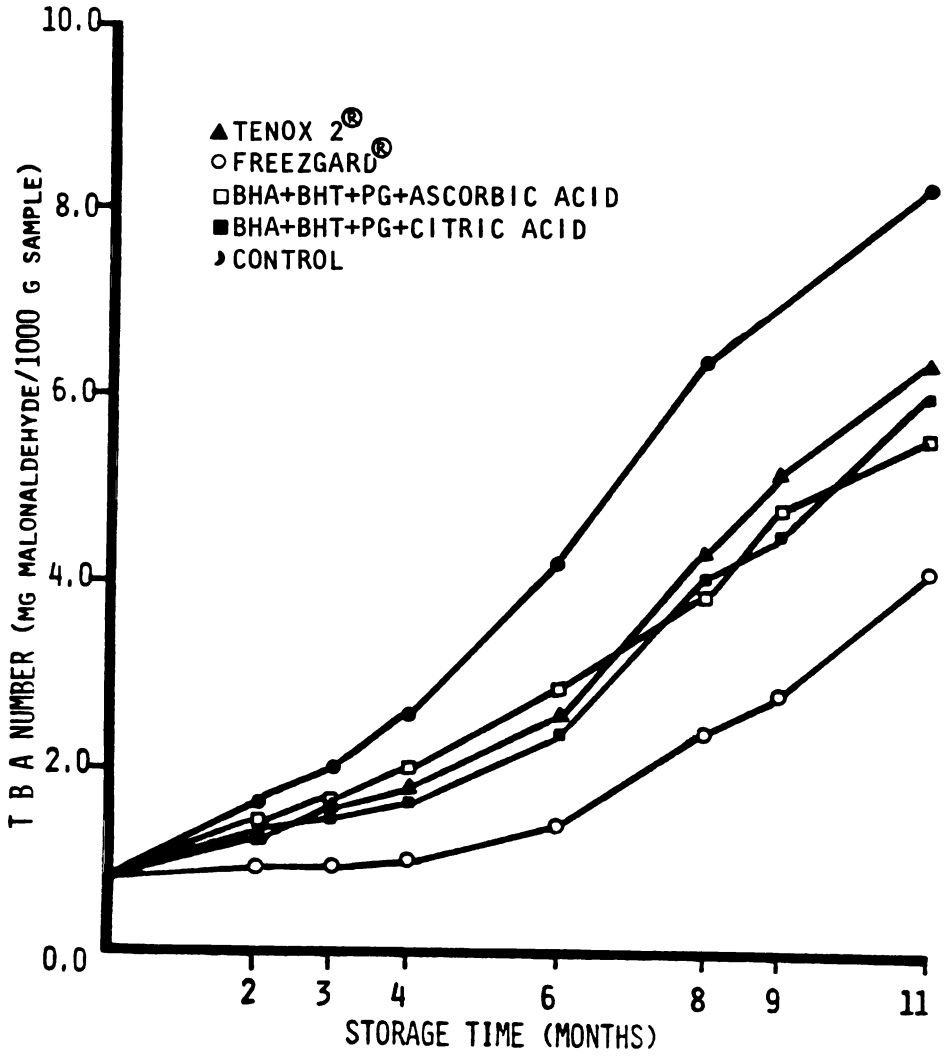


Figure 18.--Effect of antioxidants and frozen storage on TBA numbers of hand filleted carp without skin.

differences occurring at 6, 8, and 11 months of storage. TBA values were 2.64, 5.01, 7.41 for fillets with skin and 4.18, 6.41, 8.24, for fillets without skin (6, 8, and 11 months of storage, respectively).

Polyphenol antioxidant treatments on fillets with skin were ineffective because of the lower exposed muscle surface compared to skinless fillets. A slightly lower TBA number in comparison to control (but not necessarily statistically significant) were observed for skinless fillets treated with Tenox 2® and BHA + BHT + PG in presence of either ascorbic or citric acids. Freezgard® treated fillets with and without skin resulted in lowest TBA numbers among the treatments. Slight differences in TBA numbers were observed for products similarly treated between fillets with and without skin, but no significant differences were found.

Taste panel evaluations of fish loaves prepared from antioxidant treated carp fillets stored for 0, 6, 8, and 11 months at -20°C are illustrated in Figure 19. Lowest scores were recorded for Freezgard® treated samples. As explained earlier, a hedonic scale of 0 to 5 with 0 = no detectable rancidity and 5 - very rancid was used. All fillets with skin stored for 8 months were acceptable by the panel in comparison to only 6 months storage for skinless fillets (from comments on panel scoring forms). Based on an acceptable score of 2.0 or lower, phenolic

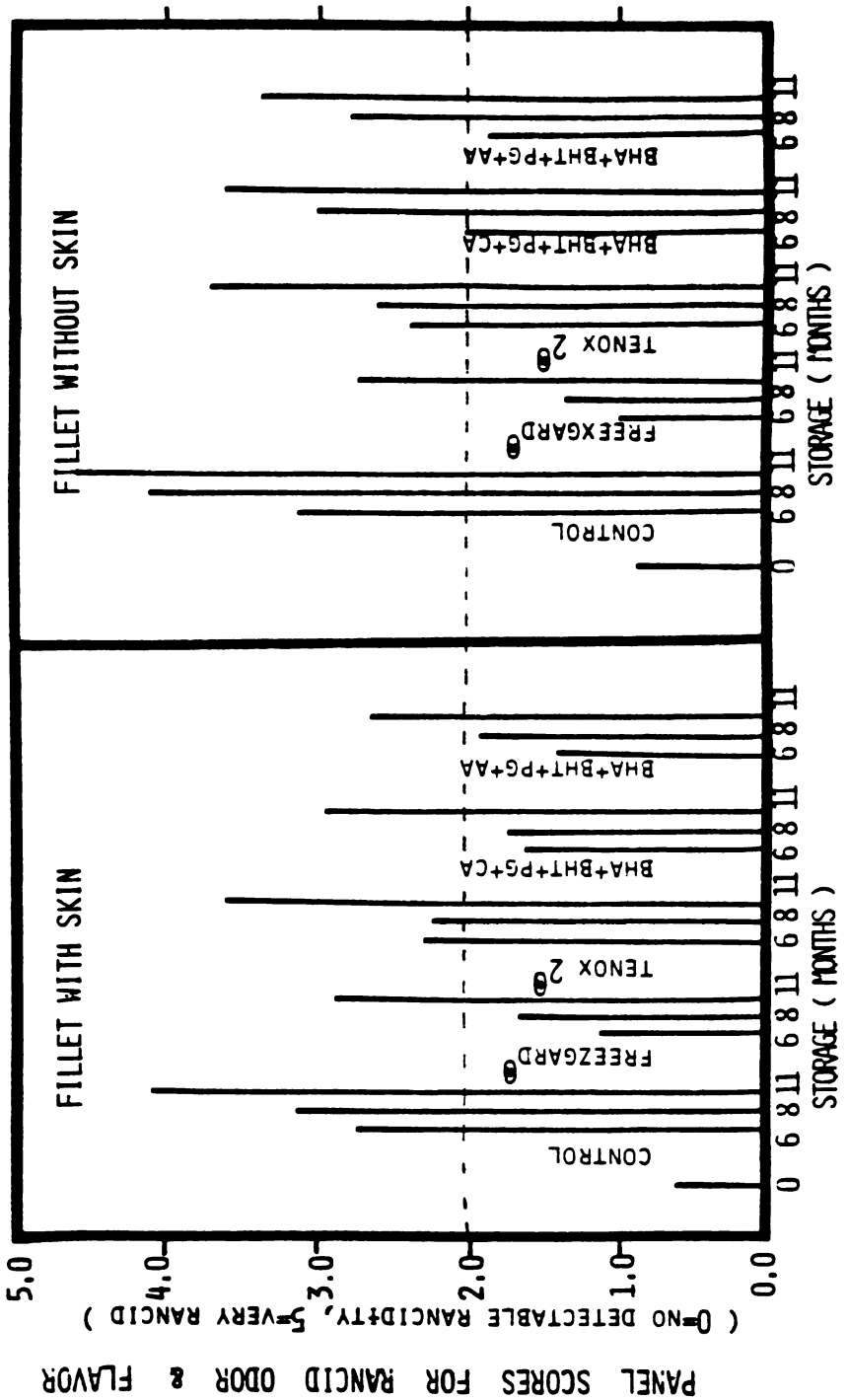


Figure 19.--Taste panel score for rancid odor and flavor of antioxidant-treated frozen carp fillets with and without skin.

antioxidants preserved acceptability for 6 months compared to 8 months for Freezgard® treated fillets.

Mullet fillets dipped for one minute in TBHQ, sodium EDTA, and ascorbic acid, singularly or in various combinations, resulted in a stable product with little rancid flavor (Deng et al., 1977). They also showed that skinless fillets stored frozen for 9 months resulted in higher TBA numbers than fillets with skin. A similar trend in the control fillets was observed in this study, but not for antioxidant treated fillets with or without skin. This may be because of the increased exposure of muscle surface which facilitate penetration of antioxidants into the skinless fillets. Results of this study also indicate that the phenolic antioxidants which are not soluble in aqueous solutions, are less effective for fish stored in the form of fillets. Those antioxidants, which resulted in lower TBA numbers in mechanically deboned minced carp, resulted in comparatively higher TBA numbers than fillets treated with Freezgard®. The increased variation in TBA numbers from phenolic antioxidant treated fillets compared to mechanically deboned minced carp (presented earlier) may be the result of nonuniform penetration of antioxidants into fillets.

Sodium erythorbate has been shown to extend shelf life of frozen Argentine hake fillets (Licciardello et al., 1980), while ascorbic acid was found to be an

effective antioxidant in extending the shelf life of frozen chub (Greig, 1967). In the above studies correlation coefficients γ = -0.767 and γ=-0.72 were reported between panel acceptance scores and TBA numbers. While a correlation coefficient between TBA numbers and sensory evaluation values of -0.74 ($p < 0.05$) was calculated in the present study, it was not possible to accurately determine the exact level between acceptable and unacceptable rancidity level based on TBA numbers. Hence, the arbitrary sensory score of 2.0 was used as an index level for acceptance which translates to acceptable frozen storage quality of 8 months for fillets with skin and 6 months for fillets without skin.

Effect of Storage Time and Antioxidants on Ratio of Unsaturated to Saturated Fatty Acids from Phospholipids

Variations in ratio of total unsaturated to saturated phospholipid fatty acids in carp fillets at the end of 3, 6, 8, and 11 months of storage and treated with various antioxidants with and without skin are listed in Table 17. Control samples of fillets with and without skin had no significant differences in their saturation ratios. However, irrespective of treatment, fillets without skin tended to retain a higher ratio of unsaturated to saturated phospholipid fatty acids. No significant differences in ratio between fillets with and without skin were found in

Table 17. Effect of time and antioxidant treatment on the ratio[1] of unsaturated to saturated phospholipid fatty acids in hand filleted carp during frozen storage.

Antioxidant Treatment	Fillets with Skin				Fillets without Skin			
	Storage Time (month)				Storage Time (month)			
	3	6	8	11	3	6	8	11
Control	2.05	1.49	1.37	1.27	2.01	1.42	1.32	1.23
Freezgard®	2.06	1.89*	1.70*	1.62*	2.04	1.86*	1.71*	1.62*
Tenox 2®	1.95	1.65	1.57	1.45	1.96	1.64	1.59	1.46
BHA + BHT + PG + AA	1.99	1.73	1.59	1.53	1.95	1.74*	1.57	1.52
BHA + BHT + PG + CA	1.98	1.71	1.62	1.42	2.01	1.68	1.55	1.49

[1]Value of ratio total unsaturated to total saturated fatty acids of phospholipids.

*Represents significant difference in a column at $p<0.05$.

products after three months of storage. After 6, 8, and 11 months of storage, significantly higher unsaturated to saturated ratios were found in Freezgard® treated products. Fillets stored for 6 months without skin and treated with BHA + BHT + PG + AA treated had a higher unsaturated to saturated ratio than all other products. These results may indicate that the advantage assumed by use of phenolic antioxidants for mechanically deboned minced carp may not be suitable for fillets due to poor penetration. Freezgard , an aqueous soluble antioxidant mixture, resulted in high unsaturation to saturation ratios and a low TBA number. This may be due to better distribution of antioxidants through the aqueous phase of the tissue and hence increased probability of contact with lipids distributed throughout the tissue. Freezgard treatments for mechanically deboned sucker were reported to be effective antioxidants (Morris and Dawson, 1979) because of its solubility in water and better distribution in tissue. In this study a similar advantage of Freezgard® was observed for fillets, while for mechanically deboned carp the advantage of distribution of the antioxidant due to its solubility in water was minimized by higher potency of phenolic antioxidants which can be evenly distributed in minced carp.

Effect of Storage Time and Treatment on Extractability of Sarcoplasmic and Myofibrillar Proteins

Water soluble and salt soluble proteins designated as sarcoplasmic and myofibrillar protein fractions were determined as mg nitrogen of protein fractions/100 g wet tissue (Figure 20). Each protein fraction extracted at the end of 3, 8, and 11 months of storage was slightly higher than the amount extracted from mechanically deboned minced carp. This indicates that intact tissues had less protein denaturation than mechanically deboned tissues during frozen storage. This change was also observed during storage of fillets with and without skin, when the fillets with skin retained a higher amount of soluble protein fractions, especially the myofibrillar fraction.

Fish receiving a Freezgard® treatment had the highest retention of soluble protein fractions in both products and least in control fillets. Phenolic antioxidant treatments resulted in intermediate level of retention of soluble protein fractions between control and Freezgard® treated fish. A very high correlation coefficient ($r = -0.93$) was found between increase in TBA numbers and decrease in retention of soluble protein fractions, indicating a probable direct impact of lipid oxidation on protein solubility and hence various other textural properties of products prepared using mechanically deboned

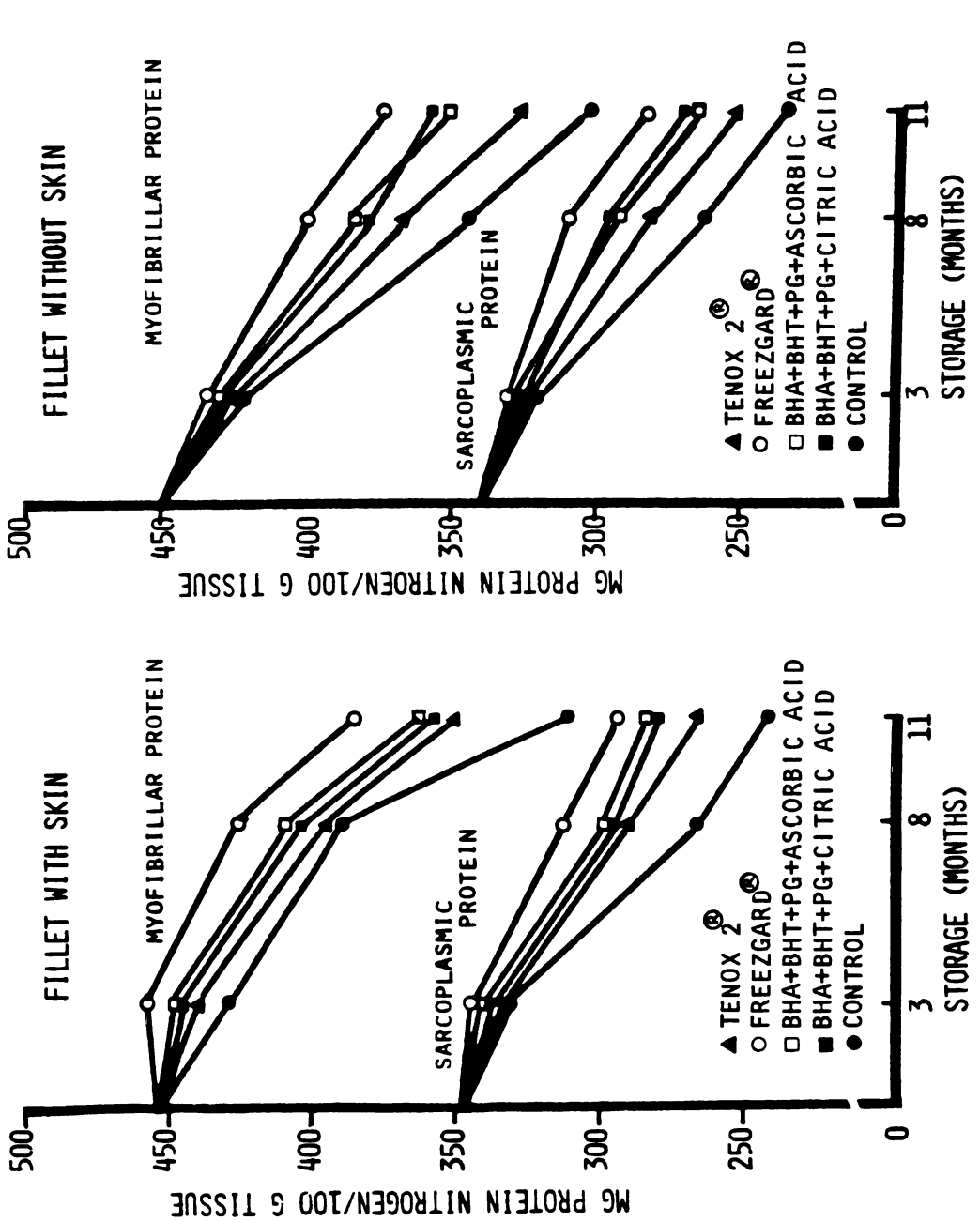


Figure 20. Effect of frozen storage and antioxidants on solubility of specific protein fractions from hand filleted carp, with and without skin.

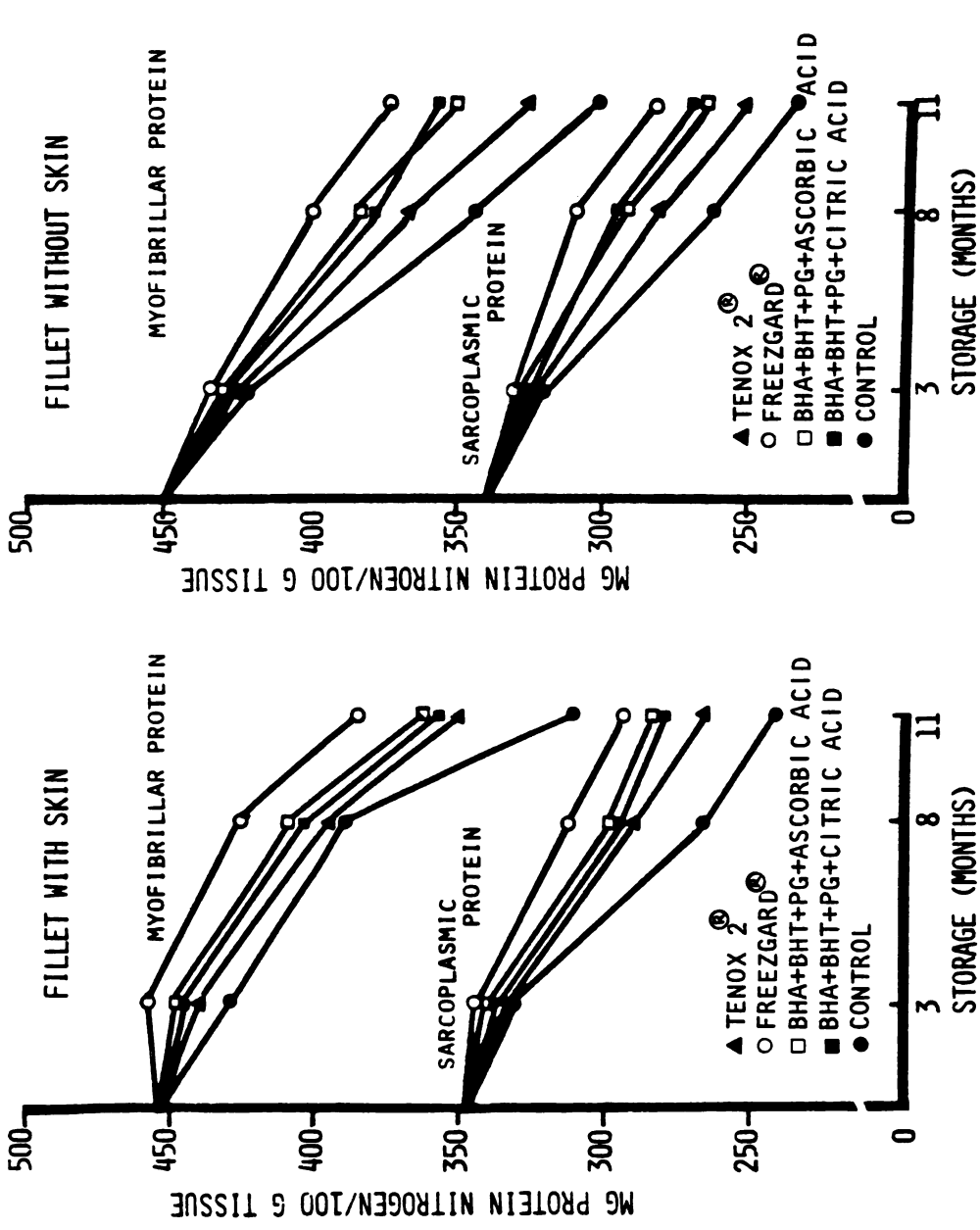


Figure 20. Effect of frozen storage and antioxidants on solubility of specific protein fractions from hand filleted carp, with and without skin.

carp.

Effect of Storage Time and Antioxidants on Shear Values of Fish Gels Prepared from Hand Filleted Carp

Mean shear values (kg force/g) of fish gels prepared from hand filleted carp with and without skin and treated with various antioxidants are listed in Table 18. Shear values decreased with storage time with maximum decrease for controls. Significantly higher shear values were found for samples of fillets with skin than without skin when treated with Freezgard®, about 10, 13, and 30 percent for the storage periods of 3, 8, and 11 months, respectively. Lower shear values were found during the first 3 months storage. A maximum of about 6.8 percent increase in shear values was recorded for fillets treated with other antioxidant treatments.

Fillets stored without skin and treated with Freezgard® had significantly higher shear values as compared to control after 11 months of storage. All samples of fillets without skin from all treatments had lower shear values than the counterparts from fillets with skin. During the first 8 months of storage, no significant differences due to treatments were observed.

Table 18. Effect of frozen storage and antioxidant treatment on shear values[1] of fish gels prepared from hand filleted carp.

Treatment	Fillets with Skin			Fillets without Skin		
	Storage (months)			Storage (months)		
	3	8	11	3	8	11
Control	1.79	1.47	1.12	1.68	1.32	0.96
Freezgard®	1.98*	1.67*	1.48*	1.79	1.43	1.30*
Tenox 2®	1.76	1.55	1.20	1.62	1.34	0.98
BHA+BHT+PG+AA	1.76	1.57	1.16	1.65	1.30	1.00
BHA+BHT+PG+CA	1.78	1.56	1.14	1.60	1.32	0.89

[1]Values are mean of 2 determinations (kg force/g sample.

*Denotes significant difference in a column ($p<0.05$).

Effect of Storage Time and Antioxidants on Water Holding Capacity of Fish Gels Prepared from Hand Filleted Carp

Percent extracted liquid from fish gels during centrifugation were used as a measure of waterholding capacity. An increased percentage of extracted liquid indicates a lower water holding capacity. These percentages for the antioxidant treated samples from fillets with and without skin at the end of 3, 8, and 11 months of storage are listed in Table 19. Freezgard® treated samples resulted in significantly higher waterholding capacity (less extractable liquids) except for fillets without skin stored for 11 months. The fillets treated with insoluble phenolic antioxidants had slightly higher or similar waterholding capacities relative to those of the control samples. In general, the fillets without skin resulted in lower waterholding capacities than the fillets with skin throughout frozen storage.

Texture analyses in this study, including protein solubility, shear force and waterholding capacities, indicated that an improved quality of frozen fish can be maintained by storing the carp as fillets, rather than as mechanically deboned minced carp (not significantly). An indirect relationship between lipid oxidation measurements and texture related measurements was observed. For example, there was an increase in TBA numbers and decrease in unsaturated to saturated fatty acid ratio of

Table 19. Effect of frozen storage and antioxidant treatment on water holding capacity[1] of fish gels prepared from carp fillets with and without skin.

Antioxidant Treatment	Fillets with Skin			Fillets without Skin		
	Storage (months)			Storage (months)		
	3	8	11	3	8	11
	(% extractable liquid)					
Control	17.8	20.0	23.1	18.4	21.7	23.8
Freezgard®	16.4*	18.3*	20.8*	17.2*	19.7*	22.3
Tenox 2®	17.5	19.6	23.0	18.4	21.2	23.6
BHA+BHT+PG+AA	17.2	19.0	22.6	18.0	20.9	22.9
BHA+BHT+PG+CA	17.3	19.2	22.1	18.1	21.2	22.8

[1] Values are mean of 2 determinations (% extractable liquid).

*Denotes significant difference in a column ($p<0.05$).

phospholipids with increase in storage time. These factors appear to be related to a decrease in solubility of protein fractions, waterholding capacity, and shear values. Cheng et al. (1979a) reported that waterholding capacity, shear values, and protein solubility values decreased with an increase in frozen storage time. The decrease in solubility of myofibrillar (salt soluble) fractions from carp tissue was higher than that of sarcoplasmic fraction. However, sarcoplasmic fraction solubility in mechanically deboned minced carp decreased rapidly during frozen storage, compared to that of fillets. This may be due to increased exposure of sarcoplasmic protein due to mechanical breaking of tissue during the deboning process. This damage is minimum during hand filleting. Those antioxidant treatments which resulted in low TBA numbers, or affected a retention of a high ratio of unsaturated to saturated phospholipids, also resulted in retentions of high amounts of soluble protein fractions, high waterholding capacity, and high shear values. This shows an indirect relationship between lipid oxidation changes and texture related parameters. Lee and Toledo (1976) and Cheng et al. (1979a) discussed the value of protein solubility, waterholding capacity, and shear values of frozen fish in predicting general textural quality of final product.

Seasonal Variations

The carp used in this study were harvested from the Saginaw Bay area of Lake Huron. Carp harvested around the 15th of each month from June 1980 through May 1981 were analyzed for proximate composition and lipid composition. During this study, due to adverse weather conditions and/or no fish harvest, sampling was not done from January through March 1981.

The mean lengths and weights of carp sampled during different months are listed in Table 20. Mean length for the sample was 67 ± 3.0 cm and weight was 3.88 ± 0.25 kg.

Seasonal Proximate Composition of Carp Flesh

Variations in proximate composition of carp flesh during the period of study are summarized in Figure 21. Average values of ash content varied from 0.86 to 1.06 percent, fat content from 7.49 to 16.8 percent, protein content from 12.1 to 17.65 percent and moisture from 69.25 to 75.43 percent. An inverse relationship was found between protein content and lipid content with the maximum protein content of about 16.92 and 17.65 percent for the carp harvested in October and December, respectively, while the lowest total protein content (about 12.1 percent) was found in carp harvested in July. Lowest total lipid content was observed for carp harvested in October and April with 7.60 and 6.45 percent, respectively. In the

Table 20. Length and weight of carp samples during various months.

Month of Harvest	Length (cm)	Weight (kg)
June 1980	69 ± 4.1	3.65 ± 0.31
July 1980	66 ± 3.3	3.71 ± 0.42
August 1980	70 ± 5.0	3.81 ± 0.20
October 1980	68 ± 1.5	4.17 ± 0.33
November 1980	70 ± 3.0	4.30 ± 0.25
December 1980	69 ± 6.1	4.00 ± 0.16
April 1981	60 ± 6.5	3.58 ± 0.17
May 1981	65 ± 5.1	3.71 ± 0.14

Note: Mean ± SD for n = 4 to 7.

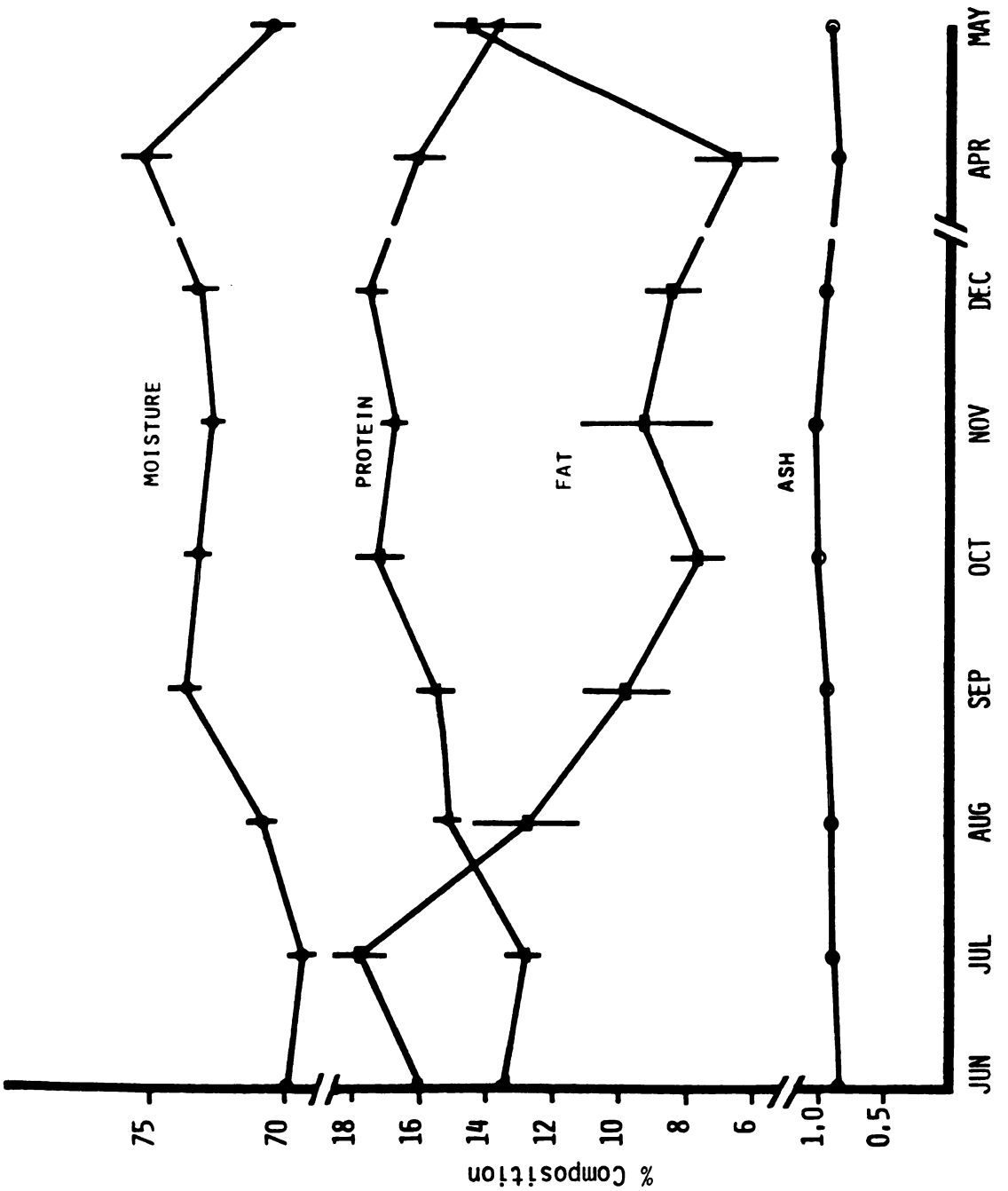


Figure 21. Seasonal variation in proximate composition of carp.

month of July the carp flesh contained the highest percentage fat accounting for 16.85 percent of the total flesh. Highest and lowest moisture levels of flesh were observed during the months of June and April accounting for about 69.25 and 75.43 percent, respectively. Generally, no significant differences in total ash content were observed due to seasonal variation.

In aquatic animals, factors such as availability of food, temperature of water, differences in diet and reproductive cycle stage are important factors that affect proximate composition of a species (Sidewell, 1976; Stansby and Lemon, 1941; Leu et al., 1981). The low levels of total lipids observed in April might be the result of post-spawning stage of carp. This minimum total lipid content was reported by Hardy and Keay (1972) and Stansby and Lemon (1941). Total lipid content of carp, and most fish in general, is dependent on size and age of the fish. By selecting fish with a small range in length and weight, variations due to differences in size can be minimized. Using this selection method, fish of about the same age were obtained and possible composition variations due to age were minimized.

Lipid Composition of Carp Flesh

Five classes of lipids consisting of phospholipids, mono, and diglycerides, triglycerides, free fatty acids, and total cholesterol were separated by TLC from flesh

lipids of carp sampled during various months. The distribution of classes of lipids during various months of analyses is shown in Table 21. The variations in total phospholipids, free fatty acids, and total cholesterol were small when expressed as percentages based on total lipid content. However, large variations in phospholipids expressed as mg/100 g wet tissue were observed with lowest value in fish harvested in April (883 mg/100 of tissue). The ratio of phospholipids to other classes of lipids were similar for each month. Significant variations in total mono and diglycerides and triglycerides from carp tissue were observed. A significantly higher triglyceride content was observed in carp harvested during the months of September through December than from other months. This may be due to variations in energy metabolism before the spawning season and also may be due to decreasing water temperatures. A similar result was implied by Watanabe and Acman (1977) regarding oysters. Since phospholipids are important in functions other than energy transformation, comparatively lesser changes in phospholipid fractions can be expected than for nonpolar or neutral lipid fractions which play an important role in energy metabolism (Stewart et al., 1972). Similar explanations can be attributed to the data on variation in phospholipid classes (Table 22).

Table 21. Seasonal variation in composition of carp flesh lipids.

Month	Phospholipids	MG + DG	TG	FFA	Total Cholesterol
June	2328 (16.32)	1900 (13.32)	9048 (53.54)*	474 (3.32)	515 (3.61)
July	2766 (17.34)	2281 (14.30)	9794 (61.39)*	234 (3.35)	578 (3.63)
August	1872 (16.12)	1544 (13.30)	7349 (63.29)*	440 (3.79)	417 (3.59)
September	1196 (15.46)	334 (4.32)*	5671 (73.30)	225 (3.30)	280 (3.62)
October	1079 (14.86)	453 (4.86)*	5348 (73.66)	230 (3.17)	251 (3.45)
November	1349 (15.07)	432 (4.82)*	6549 (73.19)	340 (3.40)	315 (3.52)
December	1138 (14.46)	549 (5.46)*	5577 (73.31)	147 (3.51)	273 (3.59)
April	882 (15.00)	566 (9.64)	3992 (67.79)	243 (4.12)	202 (3.45)
May	1996 (15.26)	1407 (10.76)	8701 (66.54)	532 (4.07)	440 (3.37)

Note: Mean of lipid fraction/100 g tissue.

Percentage of total lipid is inside parenthesis.

*Significantly lower $p<0.05$.

TABLE 22.--Seasonal variation in phospholipid composition of flesh lipids from carp harvested from Lake Huron.

Phospho-lipid[1]	June		July		August		Sept.		Oct.		Nov.		Dec.		April		May	
	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA	mg/100 g	% FA
PC	848	36.71	975	35.23	653	34.89	410	34.28	355	32.86	456	32.77	359	31.55	298	33.76	701	35.10
PE	526	22.54	610	23.06	403	21.58	251	21.02	216	20.00	298	21.60	238	20.88	201	22.84	459	22.93
SM	234	12.60	356	12.87	240	12.74	142	11.83	129	11.93	173	12.80	154	13.47	107	12.07	260	13.02
PS	238	10.15	297	10.76	195	10.41	128	10.72	128	11.87	149	11.05	136	11.83	98	11.23	203	10.17
PI	164	7.05	198	7.17	150	8.06	103	8.62	92	8.54	110	8.17	103	8.84	67	7.62	145	7.24
C	174	7.47	190	6.86	147	7.83	99	8.26	82	7.56	113	8.37	97	8.52	65	7.47	151	7.85
Others	87	3.73	113	4.05	93	4.95	65	5.27	46	4.24	72	5.20	56	4.91	44	5.01	80	4.00
Total	2328	100.00	2739	100.00	1881	100.00	1198	100.00	1048	100.00	1371	100.00	1143	100.00	880	100.00	1999	100.00

[1]Values are mg/100g tissue mean of 2 determinations.

PC = phosphatidylcholine

SM = sphingomyelin

C = cardiolipin

PE = phosphatidylethanolamine

PI = phosphatidylinositol

TABLE 23.-- Seasonal variation in fatty acid composition of flesh lipids from carp harvested from Lake Huron.

Fatty Acid[1]	June		July		August		Sept.		Oct.		Nov.		Dec.		April		May	
	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA	mg/100 g	%FA
14:0	409	2.87	747	4.68	344	2.96	227	2.93	374	5.15	458	5.12	327	4.30	119	2.02	374	2.86
15:0	79	0.54	103	0.65	71	0.61	65	0.84	33	0.45	60	0.67	37	0.49	29	0.49	81	0.62
16:0	2992	21.00	3103	19.35	1800	15.0	1166	15.02	995	13.70	1241	13.87	1148	15.09	949	16.12	2017	15.42
17:0	119	0.83	129	0.81	101	0.87	78	1.01	54	0.74	90	1.01	56	0.74	43	0.93	101	0.77
18:0	662	4.64	572	3.59	567	4.88	369	4.77	299	4.12	390	4.36	316	4.15	229	3.89	486	3.72
20:0	40	0.28	64	0.40	132	1.14	13	0.17	40	0.55	47	0.53	43	0.57	14	0.24	51	0.39
Saturates	4305	30.18	4718	29.57	3015	25.96	1918	24.79	1795	24.72	2286	25.55	1927	25.33	1383	23.49	3110	23.78
14:1	171	1.20	320	2.01	215	1.85	150	1.94	155	2.13	256	2.86	134	1.76	173	2.94	236	1.80
15:1	29	0.20	64	0.40	36	0.31	69	0.89	23	0.32	46	0.51	25	0.33	18	0.31	59	0.45
16:1	1749	12.26	2121	13.30	1399	12.05	747	9.65	719	9.90	1063	11.88	691	9.08	784	13.32	1796	13.73
17:1	228	1.60	224	1.40	131	1.13	79	1.02	54	0.74	73	0.82	59	0.78	107	1.82	118	0.40
18:1	3157	22.13	3040	19.06	2735	23.55	1942	25.10	1887	25.98	2276	25.43	1887	24.81	1127	19.14	2795	21.37
Monoenes	5334	37.39	5769	36.16	4516	38.89	2987	38.61	2838	39.08	3714	41.50	2796	36.76	2209	37.52	5004	38.27
18:2	842	5.90	1279	8.02	1226	10.56	772	9.98	946	13.03	1310	14.64	855	11.24	411	6.98	1219	9.32
18:3 + 20:1	1327	9.30	1768	11.08	1179	10.15	693	8.96	560	7.71	688	7.69	601	7.90	353	6.06	934	7.14
20:2	23	0.16	18	0.11	16	0.14	17	0.22	14	0.19	12	0.13	8	0.11	5	0.08	14	0.11
29:3	19	0.13	12	0.14	19	0.16	13	0.17	16	0.22	11	0.12	9	0.12	4	0.07	17	0.13
20:4 + 22:0	814	5.71	563	3.53	335	2.88	229	2.96	193	2.66	139	1.55	307	4.04	525	8.92	1077	8.24
20:5 ω 6	744	5.22	850	5.33	652	5.61	538	6.45	396	5.45	461	5.15	325	4.27	390	6.62	644	4.96
20:5 ω 3	20	0.14	31	0.19	26	0.22	19	0.25	18	0.25	20	0.22	11	0.14	8	0.14	29	0.22
22:4	111	0.78	162	1.02	108	0.93	89	1.15	64	0.88	70	0.78	45	0.59	80	1.36	100	0.76
22:5 ω 6	38	0.27	42	0.26	28	0.24	23	0.30	17	0.23	14	0.16	17	0.22	14	0.24	29	0.22
22:5 ω 3	188	1.32	313	1.96	206	1.77	167	2.16	176	2.46	128	1.43	208	2.76	138	2.34	304	2.32
22:6	501	3.51	417	2.61	286	2.46	272	3.52	229	3.15	96	1.07	498	6.55	366	6.22	591	4.52
Polenes	4627	32.43	5466	34.26	4081	35.14	2832	36.60	2629	36.20	2949	32.95	2884	34.91	2296	38.99	4963	37.95
TOTAL	14266		15953		11612		7737		7262		8949		7607		5888		13077	

Note: Values are mean of 2 determinants (mg/100 g tissues).

Fatty acids of total lipids from carp flesh are listed in Table 23. Twenty-five different fatty acids with carbon chain lengths of 14 to 22 were identified. The average fatty acid composition was made up of 26 percent saturated fatty acids, 38 percent monoenes, and 36 percent polyenes. The major saturated fatty acid was C16:0 (16 percent of total lipid), followed by C18:0 (4 percent) of total fatty acid content. Major monoenes were C16:1 (12 percent) and C18:1 (23 percent) of total lipid fatty acids. Major polyenes were C18:2, C18:3, C20:4, C20:5 ω 6 and C22:6 which accounted for more than 26 percent of total lipid fatty acids.

No significant differences were found in total saturated fatty acids among seasons. Total monoenes showed a decreasing trend from June to September followed by an increasing trend until December. When each major monoenoic acid C16:1 and C18:1 were considered, this decreasing trend was noticeable. Total polyenoic acids showed an increasing trend from June through October followed by a decline in November and then an upward trend. Ota and Yamada (1974) reported a decrease in C16:1, C18:1, C18:2, C18:3, acids in winter and an increase in C20:4, C20:5, and C22:6 acids in flesh lipids of Masu Salmon. They also noticed that these changes varied widely. This change in fatty acids from summer

to winter may be due to changes in specific fatty acids as identified in this study. These changes in this study may have been affected by ages of fish from 4 to 6 years, and it is known that wider variations due to season occur in young fish than old fish, other factors being equal. Fish from different habitats are known to show variations in composition due to seasonal changes. Deng et al. (1976) reported that the unsaturated fatty acid content increased from summer to winter in mullet from gulf coast areas while the unsaturated fatty acids in the same species from another area a few hundred miles away (Oakhill) was significantly different. These authors recommend a wide and extensive sampling for such a study. Total, polar, and nonpolar lipids of shortneck clam muscle have been reported to show a decrease in amount with decrease in water temperature (Ueda, 1974). A similar trend was observed in carp flesh lipids in the present study.

SUMMARY AND CONCLUSIONS

Composition and storage stability of mechanically deboned minced carp and hand filleted carp harvested from the Great Lakes were evaluated during long-term frozen storage. Emphasis was placed on changes in lipids and their fatty acids. Stability characteristics were evaluated using TBA analysis as an index of lipid oxidation. Changes in various classes of lipids and fatty acids, shear values, waterholding capacity and protein solubility were determined. Suitability of one or more antioxidant formulations for increasing the shelf life of carp during long-term frozen storage was determined. Possibility of varying storage environments as added advantages to decrease lipid oxidation was evaluated. Seasonal variation in proximate composition and detailed lipid composition of carp harvested from Lake Huron was determined. A summary of research and recommendations based on this study are as follows:

1. A higher usable meat yield of nearly 40 percent can be achieved by mechanically deboning carp as compared to 29 percent for hand deboning and mincing. This increased yield is especially advantageous when the commercially harvested carp flesh is to be incorporated

into restructured products. Mechanical deboning resulted in higher fat content of the product and a lower protein percentage of minced flesh when compared to hand deboned and minced flesh. An increase in total fat was the result of an increase in various classes of lipids including phospholipids, mono- and diglycerides, triglycerides, free fatty acids and total cholesterol. The hand deboned flesh contained a lower percent of neutral lipids compared to mechanically deboned flesh; this was the result of selective removal of depot fat during the hand deboning process. A higher level of free fatty acids was found in hand deboned flesh compared to mechanically deboned flesh. This may be the result of product temperature increasing during processing and an increased processing time during hand deboning. A comparison of carp flesh after hand deboning and mechanical deboning did not show substantial differences between various classes of phospholipids which included phosphatidyl-choline, phosphatidylserine, splingomyelin, phospha-tidylethanolamine, phosphatidylinositol, and cardiolipin. Twenty-five different fatty acids were identified inclu-ding fatty acids in the range of C14 to C22 and 0 to 6 double bonds. In both neutral and polar lipid fractions from mechanically deboned and hand deboned samples, no sig-nificant differences in presence or absence of specific fatty acids were observed. Major phospholipid fatty acids were

C14:0, C16:0, C16:1, C18:1, C18:2, C18:3, C20:1, C20:4, C20:5 ω 6, C20:5 ω 3, and C22:6.

Mechanically deboned flesh contained lower amounts of extractable sarcoplasmic, myofibrillar, and stromal protein nitrogen than hand deboned flesh. Mechanically deboned carp had lower shear values and was rated less firm by a taste panel in comparison to hand deboned carp. Mechanical deboned flesh also had lower waterholding capacity than hand deboned flesh.

2. This conclusion was based on the results from TBA analyses, changes in various lipid classes, retention of unsaturated fatty acids of phospholipids, waterholding capacity, shear values, extractability of protein, and taste panel data to identify rancidity. Tenox 2® , BHA + BHT + PG + AA and BHA + BHT + AA were satisfactory antioxidant formulations for long-term frozen storage of mechanically deboned minced carp. These antioxidants maintained satisfactory keeping quality of mechanically deboned minced carp for at least 8 months.

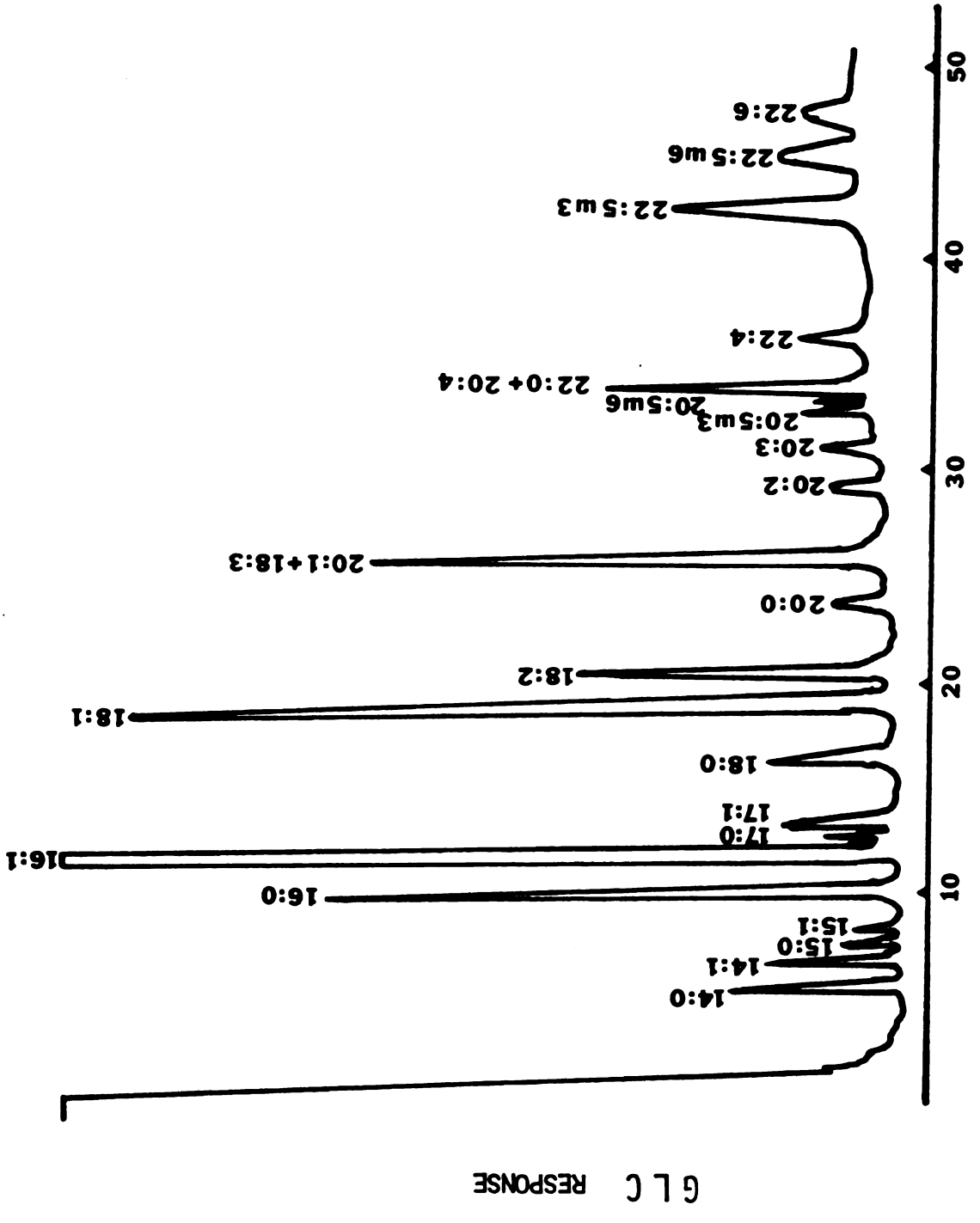
3. Air, nitrogen backflush and vacuum were used as different environments to store mechanically deboned minced carp. Nitrogen backflush treated samples had slightly lower TBA values than vacuum and air packaged samples. The cost involved in bulk storage in presence of nitrogen may limit this advantage, especially for carp which is a low value fish.

4. Stability of hand filleted carp during frozen storage was evaluated. Fillets with skin were more stable than fillets without skin. Treatment of fillets with nonaqueous soluble antioxidants resulted in low penetration and poor distribution of the antioxidant. Freezgard® was an excellent antioxidant for carp fillets during frozen storage. It was predicted from this study that shelf life of 6 and 8 months for carp fillets with and without skin can be achieved in frozen storage using Freezgard®. Overall quality of deboned minced flesh from frozen stored fillets may be superior to that of products from mechanically deboned minced frozen stored carp. This improved quality may not be advantageous from the point of view of increase in space and energy involved in frozen storage of fillets.

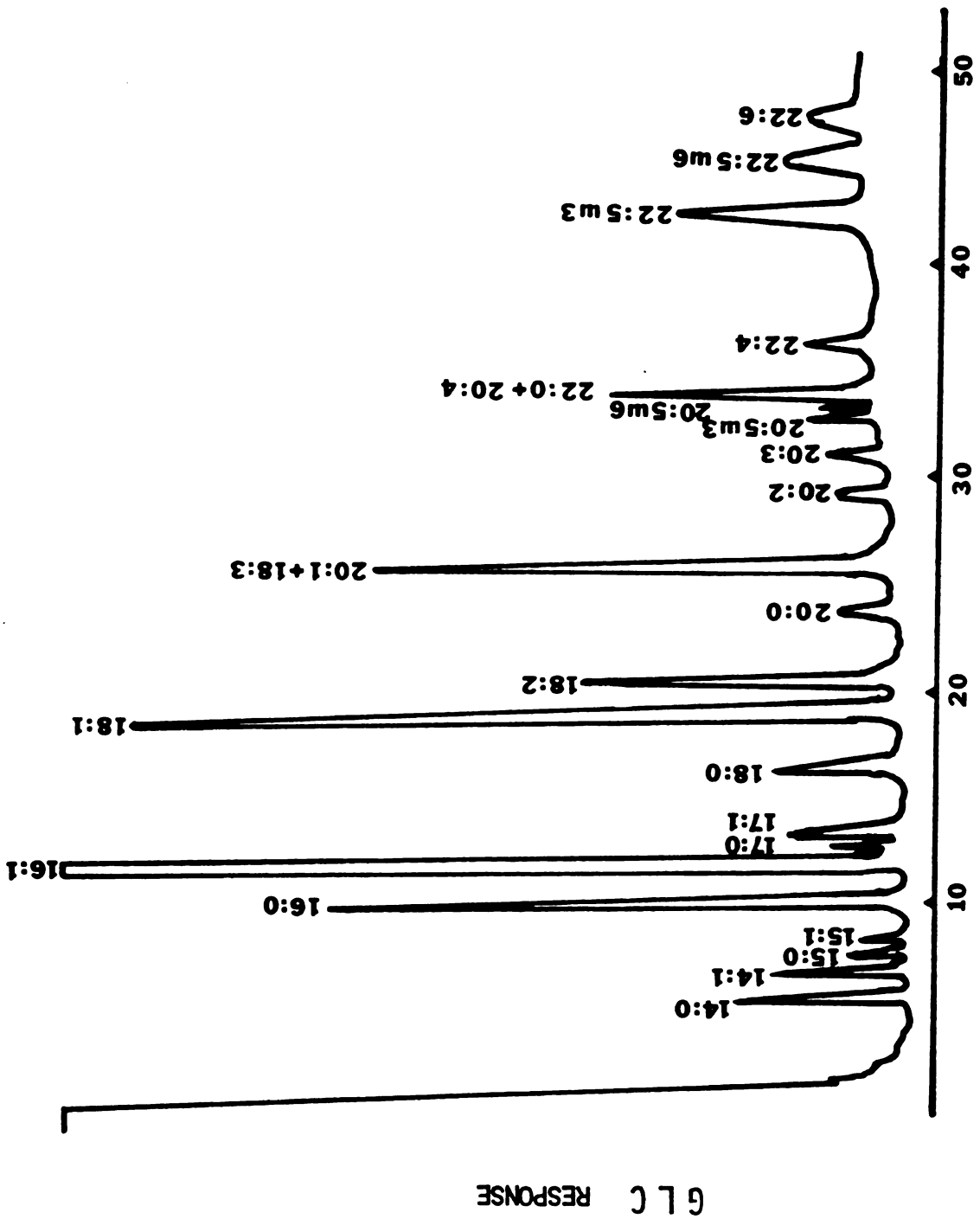
5. Seasonal variations in proximate composition and detailed total lipid fatty acid profiles were determined. Highest total fat content was observed during the months of May and July while a lower range in total lipid percentages was observed during October through April. One practical implication of this observation may be that the carp harvested during the months of May through July will require an additional process like washing of the mince to reduce total lipid content for further storage or processing. Nonpolar lipids contributed to the variation in total lipids. Ratio between type of fatty acids

and quality of fatty acids remained about the same irrespective of seasonal variation. Variations were found in total saturated fatty acids (major ones were 14:0, 16:0, and 18:0), total monoenes (major ones were 16:2 and 18:1) and total polyenes (major ones were 18:3, 20:4, 20:5 ω 6 and 22:6).

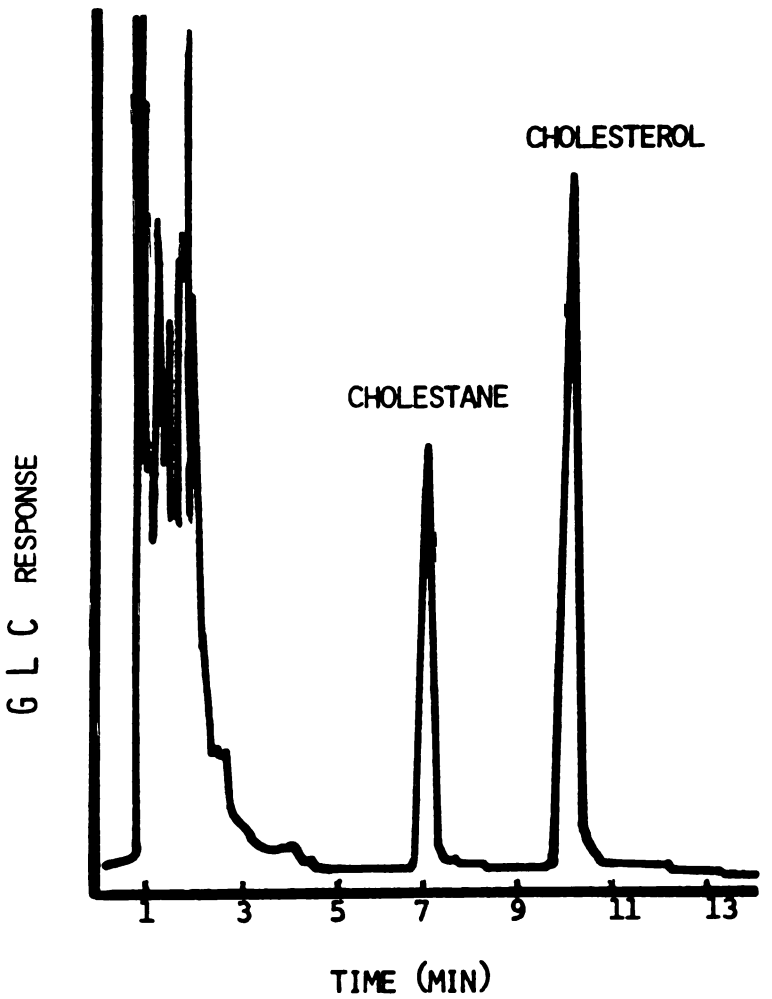
APPENDIX



Al. Typical GLC chromatogram of fatty acis from total lipids in carp flesh



Al. Typical GLC chromatogram of fatty acis from total lipids in carp flesh



A2. Typical GLC chromatogram of total cholesterol found in carp flesh lipids

EVALUATION OF RANCIDITY

NAME _____ DATE _____

Please read the fallowing before you start the evaluation.

You are being requested to evalute only the RANCID TASTE & RANCID ODOUR of the product and not the acceptability of the product it self, so disregard any biasness towards the product and rank the products based on the rancid taste and rancid odour only.

(I) You are given FOUR spiced fish loaf samples, please taste and rank them for RANCID TASTE, with a vertical line on the horrizontal line marked below representing the sample. Do rince your mouth with a sip of lemonade and a sip of water between tasting of two samples.

(II) Also you are provided with four samples in small cups covered with aluminum foils, please smell these,one at a time and rank the RANCID ODOUR using the same procedure as above.

EVALUATION OF RANCID TASTE

Sample Code:

	Rancid	Fresh
793		
500		
823		
248		

EVALUATION OF RANCID ODOUR

Sample Code:

	Rancid	Fresh
82		
38		
05		
37		

THANK You

A3. Typical evaluation form used for sensory evaluation of rancidity in frozen stored carp

EVALUATION OF TEXTURE

NAME _____ DATE _____

Please read the following before you begin evaluation

You are provided with four samples per plate, feel the sample with fingers and chew, based on touch and mouth feel, rank the samples for FIRMNESS and JUICINESS, by marking a vertical line on the appropriate horrizontal line.

eeeeeeeeee

EVALUATION OF FIRMNESS

Sample Code:

	Mushey	Firm
921	⌊_____⌋	
176	⌊_____⌋	
234	⌊_____⌋	
647	⌊_____⌋	

EVALUATION OF JUICINESS

Sample Code:

	Very dry	Very juicy
435	⌊_____⌋	
503	⌊_____⌋	
222	⌊_____⌋	
394	⌊_____⌋	

A4. Typical evaluation form used for sensory evaluation of texture.

REFERENCES

REFERENCES

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

A.O.C.S. 1974. American Oil Chemists' Official Method 12-25. In A.O.C.S. Official and Tentative Methods. Champaign, IL.

A.S.T.M. 1968a. Manual on Sensory Testing Methods. American Society for Testing and Materials. Special Technical Publication No. 434. Philadelphia, PA.

A.S.T.M. 1968b. Correlation of Subjective-Objective Methods in the Study of Odors and Taste. American Society for Testing and Materials. Special Technical Publication No. 440. Philadelphia, PA.

A.S.T.M. 1968c. Basic Principles of Sensory Evaluation. American Society for Testing and Materials. Special Technical Publication No. 433. Philadelphia, PA.

Amerine, M.A., R.M. Pangborn and E.B. Roessler. 1965. Principles of Sensory Evaluation of Food. Academic Press, N.Y.

Anderson, M.L. and M.A. Steinberg. 1964. Effect of lipid content on protein-sodium linolenate interaction in fish muscle homogenates. J. Food Sci. 29:327.

Angel, S. and Baker, R.C. 1977. A study of the composition of three popular varieties of fish in Israel, with a view towards further processing. J. Food Technol. 12:27.

Apolinario, K.M. 1975. Recovery and utilization of boneless flesh mechanically separated from tilapia, buffalo, and channel catfish. M.S. thesis, Auburn University, Auburn, AL.

Aurand, L.W., Boone, N.H., Giddings, G.G. 1977. Superoxide and singlet oxygen in milk lipid peroxidation. J. Dairy Sci. 60:363.

Awad, A., Powrie, W.D. and Fennema, O. 1969. Deterioration of freshwater whitefish muscle during frozen storage at -10°C. J. Food Sci. 34:1.

Babbitt, J.K. 1972. Minced flesh research and development at OSU Seafoods laboratory. In Oak Brook Seminar on Mechanical Recovery and Utilization of Fish Flesh, p. 49, Ed. Martin, R.E. Science and Technology, National Fisheries Institute, Washington, D.C.

Babbitt, J.K., Crawford, D.L. and Law, D.K. 1974. Quality and Utilization of Minced Deboned Fish Muscle. Second Technical Seminar on Mechanical Recovery and Utilization of Fish Flesh. Boston, MA. June 12-13. Ed. by R.E. Martin. p. 32-34.

Baker, R.C. 1970. Fish, a wasted resource. New York's Food and Life Sci. 11(4):12.

Baker, R.C., Regenstein, J.M., Raccach, M. and Darfler, J.M. 1977. Frozen minced fish; development of products from minced fish. Booklet 3, New York Sea Grant Inst., State University of N.Y., Albany, N.Y.

Benedict, R.C., Strange, E.D. and Swift, C.E. 1975. Effect of lipid antioxidants on the stability of meat during storage. J. Agr. Food Chem. 23:167.

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

Bishov, S.J. and A.S. Henick. 1972. Antioxidant effect of protein hydrolysates in a freeze-dried model system. J. Food Sci. 37:873.

Blackwood, C.M. 1974. Utilization of mechanically separated fish flesh--Canadian experience. In "FAO conference of Fishery Products, Tokyo," p. 324. Ed. Kreuger, R. Fishing News (Boods) Ltd., Surrey, England.

Blankenship, B.R., Holyday, C.E., Barnes, Jr., P.C. and Pearson, J.L. 1973. Comparison of oxygen bomb method to other methods for measuring oxidative stability of peanuts and peanut products. J. Am. Oil Chem. Soc. 50:377.

Bligh, E.G. and Regier, L. 1976. The potential and limitations of minced fish. In "The Production and Utilization of Mechanically Recovered Fish Flesh

(Minced Fish),: p. 73. Conference proceedings, Ministry of Agric., Fish and Food, Torry Research Station, Aberdeen, Scotland.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. and Physiol. 37:911.

Borton, R.J. 1969. The effects of four species of bacteria on some properties of porcine muscle protein. Ph.D. dissertation, Michigan State University, East Lansing, MI.

Bosund, J. and Ganrot, B. 1969a. Effect of precooking of Baltic herring on lipid hydrolysis during subsequent cold storage. Lebensen, Wiss. Technol. 2:59.

Bosund, J. and Ganrot, B. 1969b. Lipid hydrolysis in frozen baltic herring. J. Food Sci. 34:13.

Bottino, N.R. 1962. Nutritive value of methyl linoleate and its thermal decomposition products. J. Am. Oil Chem. Soc. 39:25.

Bremner, H.A. 1977. Storage trials on the mechanically separated flesh of three Australian mid-water fish species: 1. Analytical tests. Food Technol. Australia 29(3):87.

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

Buttkus, H. 1967. The reaction of myosin with malonaldehyde. J. Food Sci. 32:432.

Buttkus, H. 1971. The sulhydryl content of rabbit and trout myosins in relation to protein stability. Can. J. Biochem. 49:97.

Caldwell, H.M., Glidden, M.A., Kelley, G.G. and Mangel, M. 1960. Effect of addition of antioxidants to frozen ground beef. Food Res. 25:139.

Castell, C.H., Neal, W., and Smith, B. 1970. Formation of dimethylamine in stored frozen sea fish. J. Fish Res. Bd. Can. 27:1685.

Castell, C.H., Moore, B.A., Jangaard, P.M., and Neal, W.E. 1966. Oxidative rancidity in frozen stored cod fillets. J. Fish. Res. Bd. Canada 23:1385.

Carpenter, J.A. and Saffle, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29:774.

Chang, S.S. and Kummerow, F.A. 1955. An instrumental method for measuring the degree of reversion and rancidity of edible oils. J. Am. Oil Chem. Soc. 32: 341.

Chang, I.C.L. and Watts, B.M. 1952. The fatty acid content of meat and poultry before and after cooking. J. Am. Oil Chem. Soc. 29:334.

Cheng, C.S., Hamann, D.D., and Webb, N.B. 1979a. Effect of thermal processing on minced fish gel texture. J. Food Sci. 44:1080.

Cheng, C.S., Hamann, D.D. and Webb, N.B. 1969b. Effect of species and storage time on minced fish gel texture. J. Food Sci. 44:1087.

Chipault, J.R. 1962. High energy irradiation. In symposium on Foods: Lipids and their oxidation. Eds. Schultz, H.W., Day, E.A. and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CN.

Clegg, R.E., Wei, A.C. and McIntyre, R.T. 1953. Fatty acid oxidation in poultry meat extract. Poultry Sci. 32:893.

Cole, B.J. and Keay, J.N. 1976. The development of rancidity in minced herring products during cold storage. In "The Production and Utilization of Mechanically Recovered Fish Flesh (Minced Fish)," p. 66. Conference Proceedings, Ministry of Agric., Fish. and Food, Torry Research Station, Aberdeen, Scotland.

Cooney, P.A., Evans, C.D., Schwab, A.W. and Cowan, J.C. 1958. Influence of heat on oxidative stability and on effectiveness of metal inactivating agents in vegetable oils. J. Am. Oil Chem. Soc. 35:152.

Cort, W.M. 1974. Antiodixant activity of tocopherols, ascorbyl palmitate, and ascorbic acid and their mode of action. J. Am. Oil Chem. Soc. 51:321.

Coyne, F.P. 1933. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. J. Soc. Chem. 52:191.

Crawford, D.L., Law, D.K., and Babbitt, J.K. 1972. Yield and acceptability of machine separated minced flesh from some marine food fish. J. Food Sci. 37:55.

Cutting, C.L. 1969. Fish processing. In "Food Industries Manual," 20th ed., p. 213. Ed. Woolen, A. Leonard Hill Books, U.K.

Davidokova, E. and Khan, A.W. 1967. Changes in lipid composition of chicken muscle during frozen storage. J. Food Sci. 32:35.

Dawson, L.E., Uebersax, K.L. and Uebersax, M.A. 1978. Stability of freshwater sucker (Catostomus spp.) flesh during frozen storage. J. Fish. Res. Board Can. 35:253.

Deng, J.C, Orthoefer, F.T., Dennison, R.A. and Watson, M. 1976. Lipids and fatty acids in mullet (Mugil cephalus): seasonal and locational variations. J. Food Sci. 41:1479.

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

Deul, H.J., Jr., Greenberg, S.M., Calbert, C.W., Baker, R., and Fisher, H.R. 1951. Toxicological studies on isopropyl and stearyl citrates. Food Res. 16: 258.

Dingle, J.R. and Hines, J.A. 1975. Protein stability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at -5°C. J. Fish. Res. Board Can. 32:775.

Doeden, W.G., Bowers, R.H., and Engala, A.C. 1979. Determination of BHA, BHT and TBHQ in edible fats and oils. J. Am. Oil Chem. Soc. 56:12.

Dugan, L.R., Jr. 1955. Stability and rancidity. J. Am. Oil Chem. Soc. 32:605.

Dugan, L.R., Jr. 1960. Antioxidants that protect your fats and foods made with your fats. Am. Meat Inst. Found. Circ. No. 63. 7 pp.

Dugan, L.R., Jr. 1976. Chapter 4. Lipids. In "Principles of Food Science. Part I. Food Chemistry." Ed. by Owen R. Fennema. Marcel Dekker, Inc., N.Y.

Dutton, H.J., Schwab, A.W., Moser, H.A. and Cowan, J.C. 1948. The flavor problem of soybean oil. IV. Structure of compounds counteracting the effect of prooxidant metals. J. Am. Oil Chem. Soc. 25:385.

Dyer, W.J. and Dingle, J.R. 1961. Fish proteins with special reference to freezing. In "Fish as Food." Ed. Borgstrom, G., p. 275. Academic Press, New York.

Dyer, W.J. and Fraser, A. 1959. Proteins in fish muscle. 13. Lipid hydrolysis. J. Fish. Res. Board Can. 16:43.

Eckey, E.W. 1946. An oxygen-absorption method for examination of fat. Oil and Soap, 23:38.

Erickson, D.R. and Bowers, R.H. 1974. Objective determination of fat stability in prepared foods. Symposium on Objective Methods for Food Evaluation (U.S. Army Natick Laboratories and National Research Council). Boston Marriott. Newton, MA.

Eskin, N.A.M., Grossman, S. and Pinsky, A. 1977. Biochemistry of lipoxygenase in relation to food quality. CRC Crit. Rev. Food Sci. Nutr. 9:1.

Evans, C.D. 1955. Flavor evaluation of fats and oils. J. Am. Oil Chem. Soc. 32:596.

FAO/WHO Expert Committee. 1972. A review of the technological efficacy of some antioxidants and synergists. World Health Organization, Rome.

Farragut, R.N. 1972. The effect of various EDTA compounds on texture and the development of rancidity in Spanish mackeral fillets. NOAA Technical Report, NMFS, SSRF-650. Seattle, Wash.

Finne, G., Nickelson II, R., Quimby, A. and Connally, N. 1980. Minced fish flesh from nontraditional Gulf of Mexico finfish species: yield and composition. J. Food Sci. 45:1327.

Fioriti, J.A., Kanuk, M.J., and Sims, R.J. 1974. Chemical and organoleptic properties of oxidized fats. J. Am. Oil Chem. Soc. 51:219-223.

Folch, J., Lees, M., and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497.

Forss, D.A. 1973. "Progress in Chemistry of Fats and Other Lipids", Pergamon Press, N.Y.

Frankel, E.N. 1962. Hydroperoxides. In Symposium on Foods: Lipids and their oxidation. Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Freeman, I.P., Padley, F.B. and Shappard, W.L. 1973. Use of silicones in frying oils. J. Am. Oil Chem. Soc. 50:101.

Fukazawa, T., Hashimoit, Y. and Yasui, T. 1961. Effect of some proteins on the binding quality of an experimental sausage. J. Food Sci. 26:541.

Funes, J. and Karel, M. 1981. Free radical polymerization and lipid binding of lysozyme reaction with peroxidizing linoleic acid. Lipids 16:347.

Gearhart, W.M. and Stuckey, B.N. 1955. The effect of various antioxidants on the keeping quality of yellow grease. J. Am. Oil Chem. Soc. 32:287.

Georgieff, K.K. 1971. Free radical inhibitory effect of some anticancer compounds. Science 173:537.

Greene, B.E. 1971. Oxidations involving the heme complex in raw meat. J. Am. Chem. Soc. 48:537.

Greig, R.A. 1967. Extending the shelf life of frozen chub (Leucichthys hoyi) fillets through the use of ascorbic acid dips. Fishery Industrial Res. 4:23.

Hanahan, D.J., Dittmer, J.C. and Warashina, E. 1957. A column chromatographic separation of classes of phospholipids. J. Biol. Chem. 228:685.

Hanson, S.W.F. and Olley, J. 1965. Observations on the relationship between lipids and protein deterioration. In "The Technology of Fish Utilization". Ed. R. Kreuwer. Fishing News (Books) Ltd., London.

Hardy, R. and Keay, J.N. 1972. Seasonal variations in the chemical composition of Cornish mackerel, scomber scombrus (L.), with detailed reference to the lipids. J. Food Technol. 7:125.

Hao Chu, G. and Sterling, C. 1970. Parameters of texture change in processed fish: myosin denaturation. J. Texture Studies 2:214.

Hayashi, K. and Takagi, T. 1977. On the fatty acid composition of fish affected by excessive stress. Bull. Jap. Soc. Sci. Fish. 43:1189.

Hegarty, G.R., Bratzler, L.J. and Pearson, A.M. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci. 28:663.

Helander, E. 1957. On quantitative muscle protein determination. Acta Physiol. Scand. 41 (Supp. 141).

Henick, A.S., Benca, M.F., and Mitchell, J.H., Jr. 1954. Estimating carbonyl compounds in rancid fats and foods. J. Am. Oil Chem. Soc. 31:88.

Hewson, D. and Kemmish, B. 1976. Some interesting uses for minced fish in processes of reforming, upgrading, and extending. In "The Production and Utilization of Mechanically Recovered Fish Flesh (Minced Fish)," p. 84. Conference proceedings, Ministry of Agric., Fish. and Food, Torry Research Station, Aberdeen, Scotland.

Ho, S.Y. and Brown, W.D. 1966. Reactivities of lipid solvents with thiobarbituric acid. J. Food Sci. 31: 386.

Howgate, P.F. 1976. The sensory properties of minced cod and herring. In "The Production and Utilization of Mechanically Recovered Fish Flesh (Minced Fish)," p. 49. Conference proceedings, Ministry of Agric., Fish. and Food, Torry Research Station, Aberdeen, Scotland.

Ingold, K.U. 1962. Metal catalysis. In "Symposium on Foods: Lipids and their oxidation". Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Iredale, D.G. and York, R.K. 1977. Effects of chemical additives on extending the shelf life of frozen minced sucker (Catostomidal) flesh. J. Fish. Res. Board Can. 34:420.

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

Jacobson, G.A., Kirkpatrick, J.A. and Goff, H.E., Jr. 1964. A study of the applicability of a modified thiobarbituric acid test to flavor evaluation of fats and oils. J. Am. Oil Chem. Soc. 41:124.

James, A.I. 1960. Quantitative and qualitative determimination of fatty acids by gas-liquid chromatography, p. 1. In Glick, D., (ed.), "The Methods of Biochemistry Analysis." Vol. 8. Interscience Publishers Inc., N.Y.

James, A.I. and Martin, A.J.P. 1952. Gas liquid partition chromatograph. Analyst 77:915.

Jantawat, P.P. 1978. Composition and stability of lipids from mechanically processed poultry meats. Dissertation for the degree of Ph.D., Michigan State University, East Lansing, MI.

Jarvi, P.K., Lee, G.D., Erickson, D.R., and Buttkus, E.A. 1971. Determination of the extent of rancidity of soybean oil by gas chromatography compared with peroxide values. J. Am. Oil Chem. Soc. 48:121.

Jaurequi, C.A. 1978. Discoloration problems in mechanically deboned fish. Summary of New York Sea Grant Supported Thesis, p. 13. Cornell University, Ithaca, N.Y.

Johnson, O.C., Perkins, E., Sugai, M. and Kummerow, F.A. 1957. Studies on the nutritional and physiological effects of thermally oxidized oils. J. Am. Oil Chem. Soc. 34:594.

Johnston, W.R. and Frey, C.N. 1941. Autoxidation measurements on fatty oils using Barcroft-Warburg apparatus. Ind. and Eng. Chem. 13:479.

Katz, M.A., Dugan, L.R., Jr. and Dawson, L.E. 1966. Fatty acid in neutral lipids and phospholipids from chicken tissues. J. Food Sci. 31:717.

Kaunitz, H. 1962. Biological effects of autoxidized lipids. In "Symposium on Foods: Lipids and their oxidation. Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Keay, J.N. 1979. Minced fish. Torry Advisory Note No. 29. Torry Research Station, Aberdeen, Scotland.

Keeney, M. 1962. Secondary degradation products. In "Symposium of Foods: Lipids and their oxidation". Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Keller, J.D. and Kinsella, E. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. J. Food Sci. 38:1200.

Kent, M. 1975. Fish muscle in the frozen state: Time dependence of its microwave dielectric properties. J. Food Technol. 10:91.

Killeffer, D.H. 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22:140.

King, F.J. 1978. Past, present, and future uses of minced fish in the United States. Presented at the Aberdeen (Ill.) conference on the utilization of minced fish, Aberdeen, Scotland.

King, F.J. 1972. Characteristics, uses, and limitations of comminuted fish during storage at -5°C. J. Fish. Res. Board Can. 32:775.

King, F.J. and Carver, J.H. 1971. How to use nearly all the ocean's food. Commercial Fish. Rev. 32:12.

King, F.J., Anderson, M.L., and Steinberg, M.A. 1962. Reaction of cod actomyosin with linoleic and linolenic acids. J. Food Sci. 17:363.

King, N.A. 1981. Influence of antioxidant and package environments on stability of turkey patties. M.S. Thesis, Michigan State University, East Lansing, MI.

Kinsella, J.E., Shimp, J., Mai, J., and Weihrauch, J. 1977. Fatty acid content and composition of freshwater finfish. J. Am. Oil Chem. Soc. 54: 424.

Klinger, S.D. 1974. Flavor and acceptability of preroasted duck. M.S. Thesis, Purdue University, West Lafayette, IN.

Kovacs, M.I.P., Anderson, W.E., and Ackman, R.G. 1979. A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. J. Food Sci. 44:1299.

Kraybill, H.R., Beadle, B.W., Vibrans, F.C., Swartz, V., Wilder, O.H.W., and Rezabeck, H. 1948. Studies on antioxidants. I. AMI 72 a superior antioxidant for animal fats. Am. Meat Inst. Found. Bull. No. 2. 7 pp.

Kudo, G., Okada, M., and Miyauchi, D. 1973. Gel-forming capacity of washed and unwashed flesh of some Pacific coast species of fish. MFR paper 1021, Marine Fish. Rev. 35:10.

Kuksis, A. 1965. Gas-liquid chromatography of glycerides. J. Am. Oil Chem. Soc. 42:269.

Kummerow, F.A. 1962. Toxicity of reheated fats. In "Symposium on Foods: Lipids and their oxidation. Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Kwon, T.W., Munzel, D.B. and Alcott, M.S. 1965. Reactivity of malonaldehyde with food constituents. J. Food Sci. 20:808.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Critical Review in Food Technology 2:355.

Labuza, T.P., Silver, M., Cohn, M., Heidelbaugh, N.D., and Karel, M. 1971. Metal-catalyzed oxidation in the presence of water in foods. J. Am. Oil Chem. Soc. 48:527.

Lancaster, E.B., Bitner, E.D., and Beal, R.E. 1956. Recording apparatus for measuring oxygen absorption. J. Am. Oil Chem. Soc. 33:36.

Lanier, T.C. and Thomas, F.B. 1978. Minced fish: its production and use. Public. UNC-SG-78-08, Sea Grant College Program, University of North Carolina, Raleigh, N.C.

Lea, C.H. 1962. The oxidative deterioration of food lipids. In "Symposium on Foods: Lipids and their Oxidation." Ed. Schultz, H.W., Day, E.A., and Sinnhuber, R.O., p. 3. AVI Publ. Co., Westport, CT.

Lea, C.H. 1960. Antioxidants in dry fat systems: Influence of the fatty acid composition of the substrate. J. Sci. Food Agric. 11:143.

Lea, C.H. and Swoboda, P.A.T. 1958. A new procedure for the determination of volatile carbonylic substances in autoxidizing edible fats. Chem. and Ind. 40:974.

Lee, C.M. and Toledo, R.T. 1977. Degradation of fish muscle during mechanical deboning and storage with emphasis on lipid oxidation. J. Food Sci. 42:1646.

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

Leu, S.S., Jhaveri, S.N., Karakoltsidis, P.A., and Constantinides, S.M. 1981. Atlantic mackerel (Scomber scombrus, L.): Seasonal variation proximate composition and distribution of chemical nutrients. J. Food Sci. 46:1635.

Licciardello, J.J., Ravesi, E.M. and Allsup, M.G. 1980. Extending the shelf life of frozen Argentine hake. J. Food Sci. 45:1312.

Lindsay, R.C. 1975. A summary of the effect of antioxidants and film packaging on oxidative quality of fish during long term frozen storage. A Manuscript summarizing M.S. thesis by Steven, J. Teets, University of Wisconsin, Madison, WI.

Love, J.D. and Pearson, A.M. 1971. Lipid oxidation in meat and meat products--a review. J. Am. Oil Chem. Soc. 48:547.

Love, R.M. 1968. Ice formation in frozen muscle. In "Low Temperature Biology of Food Stuffs." J. Hawthorn and E.J. Rolfe, Pergamon Press, Oxford, London.

Love, R.M. and Abel, G. 1966. The effect of phosphate solutions on the denaturation of frozen cod muscle. J. Food Technol. 1:323.

Lundberg, W.O. 1962a. Autoxidation and antioxidants. Vol. II. Wiley-Interscience, N.Y.

Lundberg, W.O. 1962b. Mechanisms (of lipid autoxidation). In "Symposium on Foods: Lipids and their oxidation. Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Lundberg, W.O. 1961. Autoxidation and antioxidants. Vol. I. Wiley-Interscience, N.Y.

Machlin, L.J. 1962. Role of antioxidants in the biological fate of lipids. In "Symposium on Foods: Lipids and their oxidation". Eds. Schultz, H.W., Day, E.A., and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

Mai, J. and Kinsella, J.E. 1979. Changes in lipid composition of cooked minced carp (Cyprinus carpio) during frozen storage. J. Food Sci. 44:1619.

Manohar, S.V., Rigby, D.L. and Dugal, L.C. 1973. Effect of sodium tripolyphosphate on thaw drip and taste of fillets of some fresh water fish. J. Fish Rev. Board Can. 30:685.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50: 387.

Matsuo, N. 1962. Nutritional effects of oxidized and thermally polymerized fish oils. In "Symposium on Foods: Lipids and their oxidation". Eds. Schultz, H.W., Day, E.A. and Sinnhuber, R.O. The AVI Publishing Co., Inc., Westport, CT.

McCarthy, R.D. and Duthie, A.H. 1962. A rapid quantitative method for the separation of free fatty acids from their lipids. J. Lipid Res. 3:117.

McGinnis, G.W. and Dugan, L.R., Jr. 1965. A rapid low temperature method for preparation of methyl esters of fatty acids. J. Am. Oil Chem. Soc. 42:305.

McKerrigan, A.A. 1957. The estimation of carbonyl compounds as a criterion on flavor deterioration in edible fats containing antioxidants. Antioxidants in Foods Symposium. Low Temperature Research Station, Cambridge, MA.

McMurray, W.C. and Magee, W.O. 1972. Phospholipid metabolism. Ann. Rev. Biochem. 41:149.

Mehlenbackner, V.C. 1960. The analysis of fats and oils. Garrard Press. Champaign, IL. (pp. 571-575).

Metcalfe, L.D., Schmitx, A.A. and Pelka, J.R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514.

Mickelberry, W.C. 1970. Effect of in vivo injection of antioxidants on turkey fat stability. Poultry Sci. 49:355.

Miyauchi, D. 1972. Procedure for preparing modified minced-fish blocks. Informational report, U.S. Dept. of Commerce, Pacific Fishery Products Center, Seattle, WA.

Miyauchi, D., Patashnik, M. and Kudo, G. 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 Steinberg, M. 1971. Machine separation of edible flesh from fish. Fish. Industrial Res. 6(4): 165.

Moledina, K.H., Regenstein, J.M., Baker, R.C. and Steinkraus, K.H. 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.

Moorjani, M.N., Baliga, B.R., Vijayaranga, B. and Lahiry, N.L. 1962. Post-rigor changes in nitrogen distribution and texture of fish during storage in crushed ice. Food Technol. 16:80.

Morris, D.M. and Dawson, L.E. 1979. Storage stability of mechanically deboned sucker (Catastomidae) flesh. J. Food Sci. 44:1093.

Morrison, W.R. 1964. A fast simple and reliable method for the micro-determination of phosphorus in biological materials. Analytical Biochem. 7:218.

Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. J. Lipid Res. 5: 600.

Moser, H.A., Evans, C.D., Cowan, J.C. and Kwolek, W.F. 1965. A light test to measure stability of edible oils. J. Am. Oil Chem. Soc. 42:30.

Naidu, Y.M. and Dawson, L.E. 1983. Influence of packaging film, environment and BHA on stability of turkey products in frozen storage. Unpublished data.

Nakayama, T. and Sato, Y. 1971. Relationships between binding quality of meat and myofibrillar proteins. 3. Contribution of myosin and actin to rheological properties of heated minced-meat gel of Texture Studies 2:75.

Nelson, G.J. 1975. Fractionation of phospholipids, p. 70. In E.G. Perkins (Ed.), "Analysis of Lipids and Lipoproteins." Americal Oil Chemistry Society, Champaign, IL.

Noble, J. 1974. New protein source from the sea. Food Industries of South Africa 16:70.

Noble, J. 1973. Retrieved meat waste to build new foods. Food Envineering 45:76.

Nolen, G.A. 1972. Effects of fresh and used hydrogenated soybean oil on reproduction and teratology in rats. J. Am. Oil Chem. Soc. 49:688.

O'Brien, J.S. and Benson, A.A. 1964. Isolation and fatty acid composition of plant sulfolipid and galactolipids. J. Lipid Res. 5:432.

Olcott, H.A. 1974. Round table on prediction of fat stability symposium on objective methods for food evaluation (U.S. Army Natick Lab), Newton, MA.

Olley, J. and Lovern, J.A. 1960. Phospholipid hydrolysis in cod flesh stored at various temperatures. J. Sci. Food Agric. 11:644.

Orr, C.H. and Callen, J.E. 1958. Separation of poly-unsaturated fatty acid methyl esters by gas chromatography. J. Am. Chem. Soc. 80:249.

Ostrander, J. and Dugan, L.R., Jr. 1961. A rapid quantitative lipid extraction method. Am. Meat Institute Foundation Bull. No. 30.

Ota, T. and Yamada, M. 1974. Seasonal variation in the lipids of Masu salmon parr during the life in fresh water. Bull. Jap. Soc. Sci. Fisheries. 40:699.

Parker, F. and Peterson, N.F. 1965. Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatograms. J. Lipid Res. 6: 455.

Patashnik, M., Miyauchi, D. and Kudo, G. 1974. Controlling bone particle content in minced fish muscle. MFR paper 1079, Marine Fish. Rev. 36(8):37.

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

Peng, C.Y. and Dugan, L.R., Jr. 1965. Composition and structure of phospholipids in chicken muscle tissues. J. Am. Oil Chem. Soc. 42:533.

Phillips, J.A. and Vail, G.E. 1967. Effect of heat on fatty acids. J. Am. Diet. Assoc. 50:116.

Pigott, C.M. and Shenouda, S.Y.K. 1975. Lipid-protein interactions during aqueous extraction of fish protein: actin-lipid interaction. J. Food Sci. 40:523.

Pohle, W.D., Gregory, R.L. and Giessen, B.V. 1964. Relationship of peroxide value and thiobarbituric acid value to development of undesirable flavor characteristics in fats. J. Am. Oil. Chem. Soc. 41: 649.

Poling, C.E., Warner, W.D., Mone, P.E. and Rice, E.E. 1962. The influence of temperature, heating time, and aeration upon the nutritive value of fats. J. Am. Oil Chem. Soc. 39:315.

Price, J.F., Rippen, T.E., Gartner, R.M., Dawson, L.E., Bonilla, E.F., Booren, A.M. and Reynolds, A. 1981. Increasing the economic value of Michigan's commercial fishery through the utilization of carp. Final project report. Project No. R/CF8.

Privett, O.S. 1959. Autoxidation and autoxidative polymerization. J. Am. Oil Chem. Soc. 36:507.

Radin, N.A. 1969. Preparation of lipid extracts, p. 245. In Lowenstein, J.M. (Ed.). "Methods of Enzymology", Vol. 14, Lipids, Academic Press, New York.

Raja, K.C.M., Moorjani, M.N. 1971. Nucleotides-A study of their degradation in fish under various conditions and its impact on flavour. J. Food Sci. and Technol. 8(1):3.

Rawls, H.R. and van Santen, P.J. 1970. A possible role for singlet oxygen in the initiation of fatty acid autoxidation. J. Am. Oil Chem. Soc. 47:121.

Reseck, J. and Waters, M. 1979. Acceptability, stability of underutilized fish species suggest commercial potential. Food Prod. Develop. 13(5):46.

Rippen, T.E. 1982. Selected treatments and properties of mechanically deboned carp (Cyprinus carpio) flesh. M.S. thesis, Michigan State University, East Lansing, MI.

Rouser, G., Siakotos, N. and Fleisher, S. 1966. Quantitative analysis of phospholipid by thin layer-chromatography and phosphorus analysis of spots. Lipids 1:85.

Samejima, K., Hashimoto, Y.Y., Yasul, U. and Fukazawa, T. 1969. Heat gelling properties of mysoin, actin, actomyosin and myosin-subunits in a saline model system. J. Food Sci. 34:242.

Sato, J. and Herring, H.K. 1973. The chemistry of warmed-over flavor in cooked meats. In Proc. of 26th Ann. Reciprocal Meat Conference of the American Meat Science Association. The Pennsylvania State University, University Park, PA. June 17-20.

Satterlee, L.D., Froning, G.W. and Janky, D.M. 1971. Influence of skin content on composition of mechanically deboned poultry meat. J. Food Sci. 36:979.

Schaich, K.M. and Kearel, M. 1975. Free radicals in lysozyme reacted with peroxidizing methyl linoleate. J. Food Sci. 40:456.

Scholz, R.G. and Ptak, L.R. 1966. A gas chromatographic method for measuring rancidity in vegetable oils. J. Am. Oil Chem. Soc. 43:596.

Seino, M., Watanabe, S., Nihongi, T. and Nagai, T. 1973. Influencing of operating conditions on determining of fatty acid methyl esters by gas chromatography. J. Am. Oil Chem. Soc. 50:335.

Sheppard, A.J., Hubbard, W.D. and Prosser, A.R. 1974. Evaluation of eight extraction methods and their effects on total fat and gas liquid chromatographic fatty acid composition analyses of food products. J. Am. Oil Chem. Soc. 51:416.

Sherwin, E.R. 1968. Methods for stability and antioxidant measurement. J. Am. Oil Chem. Soc. 45:632A.

Sherwin, E.R. 1972. Antioxidants for food fats and oils. J. Am. Oil Chem. Soc. 49:468.

Shewan, J.M. 1950. Improving the quality of white fish by the use of gas storage. Fish News No. 1946, p. 14.

Shono, T. and Toyomizu, M. 1973. Lipid alteration in fish muscle during cold storage--I. Expression of lipid hydrolysis and oxidation in Jack mackerel muscle boned on decrease in C22:6 acid. Bull. Jap. Soc. of Sci. Fisheries 38:411.

Shono, T. and Toyomizu, M. 1971. Changes in fatty acids constituting lipids in fish muscle during storage at low temperature (5^{o}C) decrease rate of C22:6 acid as a criterion for the oxidative deterioration of lipids. Bull. Jap. Soc. Sci. Fish. 37:912.

Sidwell, V.D. 1976. Chemical and nutritive composition of mullet. In "Economics, Biology, and Food Technonogy of Mullet." Ed. Cato, J.C. and McCullough, W.E. Florida Sea Grant Program. Report No. 15.

Sikorski, Z., Olley, J. and Kostuch, S. 1976. Protein changes in frozen fish. Critical Reviews in Food Sci. and Nutrition.

Sims, R.J. and Hilfman, L. 1956. Stabilization of edible animal fats during rendering. J. Am. Oil Chem. Soc. 33:381.

Skipski, V.P., Peterson, R.F. and Barclay, M. 1962. Separation of phosphatidylethanolamine, phosphatidyl serine and other phospholipids by thin-layer chromatography. J. Lipid Res. 3:467.

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

Stansby, M.E. and Lemon, J.J. 1941. Resource Representation. U.S. Fish and Wildlife Serv. No. 1, p. 1.

Stansby, M.E. and Olcott, H.S. 1963. Composition of fish in Industrial Fishery Technology. Stansby, M.E., Ed. Reinhold, N.Y., p. 339.

Steel, R.G. and Torrie, H.J. 1960. "Principles and Procedures of Statistics." McGraw-Hill, New York.

Steinberg, M.A. 1975. USDA issues detailed report on uses of minced fish. Quick Frozen Foods Intern. 16(3):115.

Stemmber, M.H. 1974. German Patent No. 2220685. Food Technol. 28:99.

Stewart, J.E., Hornett, G.W. and Arie, B. 1972. Effect of temperature, food and starvation on several physiological parameters of lobster hormonus americanus. J. Fish. Res. Bd. Canada, 19:439.

Stuckey, B.N. 1962. Antioxidants. In "Symposium on Foods: Lipids and their oxidation". Eds. Schultz, H.W., Day, E.A. and Sinnhuber, R.O. The AVI Publishing Co., Westport, CT.

Swift, C.E. 1965. The emulsifying properties of meat proteins. In "Proc. Meat Ind. Research Conf.", p. 78. Am. Meat Sci. Assoc. and Am. Meat Inst. Foundation, Chicago, IL.

Takama, K. 1974. Changes in flesh lipids of fish during frozen storage. V. Accelerative substances of lipid oxidation in the muscle of the rainbow trout. Bull. Faculty Fisheries. Hokkaido. Univ. Hokkaido Daigaku, Sho. 25:256.

Takama, K., Zama, K. and Igaroshi, H. 1967. Changes in flesh lipids of fish during frozen storage: flesh lipids of blue fin tuna. Hakkaido Daigoku Suidan Gakubu Kenkyu Sho. 18:240.

Tappel, A.L. 1961. Biocatalysts: lipoxidase and hematin compounds. In "Autoxidation and Antioxidants", Vol. I. Ed. Lundberg, W.O. Interscience Publishers, N.Y.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15:602.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R., Jr. 1962. The chemistry of the -thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39:34.

Tarladgis, B.G. 1961. An hypothesis for the mechanism of the heme-catalyzed lipid oxidation in animal tissues. J. Am. Oil Chem. Soc. 38:479.

Tarladgis, B.G. and Watts, B.M. 1960. Malonaldehyde production during the controlled oxidation of pure, unsaturated fatty acids. J. Am. Oil Chem. Soc. 37:403.

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

Teeny, F.M. and Miyauchi, D. 1972. Preparation and utilization of frozen blocks of minced black rockfish muscle. J. Milk and Food Technol. 35:414.

Trautman, J.C. 1970. Method of extracting salt soluble protein from post-rigor meat. U.S. Patent 3,523,800. Food Technol. 23:101.

Tasi, R., Cassens, R.G. and Briskey, E.J. 1972. The emulsifying properties of purified muscle proteins. J. Food Sci. 37:286.

UGLRC (Upper Great Lakes Regional Commission) Report. 1978. Increasing the economic value of Upper Michigan's commercial fishery. Project No. 10620305, Michigan State University, East Lansing, MI.

Uri, N. 1961. Autoxidation and antioxidants, Vol. I. Ed. Lundberg, W.O. Interscience Publishers, Inc., N.Y.

U.S.D.A. 1960. Regulations governing the meat inspection of the U.S.D.A. Meat Inspection Division of the U.S.D.A. Fed. Reg. 24:9368.

Venkataraman, R., Solonki, K.K. and Kandorais, M.K. 1968. Seasonal variation in the chemical composition of pomfrets-1 block pomfret (Parastromateus niger), Fishery Technol. 5:113.

Vianni, R. 1980. Photoxidation studies on soybean oil. Ph.D. dissertation, Michigan State University, E. Lansing, MI.

Watanabe, T. and Acman, R.G. 1977. Effect of storage on lipids and fatty acids of oysters. J. Inst. Can. Sci. Technol. 10:40.

Watts, B.M. 1954. Oxidative rancidity and discoloration in meat. In "Advance in Food Research", 4:1. Academic Press, Inc., N.Y.

Webb, N.B. 1974. Emulsion technology. Proc. Meat Industry Res. Conf. p. 1. Am. Meat Sci. Assoc. and Am. Meat Inst. Foundation. Chicago, IL.

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

Weiss, T.J. 1970. "Food Oils and Their Uses." AVI Publishing Co., Westport, CT.

Witting, L.A., Nishidak, T., Johnson, O.C. and Kummerow, F.A. 1957. The relationship of pyridoxine and riboflavin to the nutritional value of polymerized fats. J. Am. Oil Chem. Soc. 34:421.

Wong, J., Lau, Y.C. and Yamamoto, M. 1978. Mechanical fish deboners: influence of perforation sizes on bone content and texture of minced fish flesh. J. Food Sci. 43:807.

Yu, T.C. and Sinnhuber, R.O. 1967. An improved 2-thiobarbituric acid (TBA) procedure for the measurement of autoxidation in fish oils. J. Am. Oil Chem. Soc. 44: 256.

Yu, T.C. and Sinnhuber, R.O. 1964. Further observations on the 2-thiobarbituric acid method for the measurement of oxidative rancidity. J. Am. Oil Chem. Soc. 41:540.

Zapata, J.F. 1978. The functional properties of the mechanically deboned sucker (Catostomidae family) flesh. M.S. thesis, Michigan State University, East Lansing, MI.