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By

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ABSTRACT

SUCKER (CATOSTOMUS COMMERSONI) PASTE PRODUCTS AND THEIR QUALITY STABILITY DURING FROZEN STORAGE

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Mechanically deboned sucker flesh was used to study the effect of washing technique on the extractability of myofibrillar proteins, gel forming ability as well as the lipid composition, cholesterol level and thiobarbituric acid (TBA) values; and the lipid stability on fish paste products stored at -23^{o}C for six months.

The washing technique reduced the water soluble constituents, G-actin, tropomyosin, unbound lipids, free cholesterol and TBA values; and increased the relative concentration of myosin, M-line protein, C-protein, α-actinin, β-tropomyosin, and α-tropomyosin. The extractability of myofibrillar proteins decreased as the storage time increased. A significant correlation was found between the myofibrillar protein extractability and the gel forming ability. The gel strength of the cooked fish balls are directly related to the amount of total myosin, C-protein, and β-tropomyosin that are present in the fish pastes.

Preheating of fish pastes and addition of sodium hexametaphosphate (SHMP), monosodium glutamate (MSG) and sucrose which greatly improved the quality stability and

retarded the rate of phospholipid hydrolysis. Addition of NaCl which promoted lipid oxidation resulted in higher TBA values than those with no NaCl added. Presence of SHMP retarded the lipid oxidation which was probably promoted by NaCl and metal ions resulting in low TBA values. The rate of increase in proportion of neutral lipids and hydrolysis of phospholipids is faster in raw fish pastes than in cooked fish balls; among the raw fish pastes, it is slower in sample containing SHMP than those containing NaCl. Cholesterol levels decreased slightly but were not significantly different among treatments. The quality of both raw and cooked fish pastes was considered acceptable with TBA values below 2. The fish balls were evaluated as acceptable with no detectable oxidative rancidity after six months of storage at $-23^{o}C$.

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INTRODUCTION

Fish are a widely distributed source of high-quality dietary proteins, which have the potential to meet the world's needs as population increases. According to the FAO (1979) annual report, the world total catches of freshwater fish in inland water was 6,100 metric tons (MT) in 1975, 5,896 MT (1976, 6,043 MT (1977), 5.786 MT (1978) and 6,039 MT in 1979. Two-thirds of the total fish caught were in Asia, one-sixth were in Africa, the rest were in USSR, Europe, South America and North America in decreasing order of the amount of the total harvest.

Fishery statistics of the U.S.A. indicated that harvested from Lake Huron in 1976 were 2.2 million pounds worth $771,171 which represents an increase of 301,900 pounds and $141,606. Major species such as carp, catfish, suckers, whitefish, and yellow perch increased slightly in landing (Pileggi and Thompson, 1980).

Suckers harvested in 1976 totaled only 126,800 pounds with an economic value of $11,195 from Lake Huron. However, these figures could probably be increased by expanding fishing effort and seaching for new product development as well as for the possibility of increased consumer market.

Bay Port Fishery Company has been attempting to expand the market for frozen sucker surimi since 1979. Now, the surimi that is prepared by blending an antioxidant FreezGard into the minced sucker flesh is available in Detroit super markets.

The manufacture of gel-type fish products has been widely practiced in Asia, especially in Japan and the Southern provinces of China. In late 1940's, the first mechanical deboning machine was applied in Japan in an attempt to increase the number of species used in the production of surimi, the basic material used for the manufacture of Kamaboko, fish balls and similar types of comminuted fish products. The magnitude of the annual production of surimi is over one million metric tons in Japan (Wong et al., 1978). In Taiwan, the production of minced fish products reached 17,625 metric tons in 1976; and it is expected that minced fish products may become the largest item in processed fish industry (Hwang and Jeng, 1979).

The increased use of mechanically deboned fish flesh into reconstructed fish products has received world-wide attention, especially with respect to the total yield of recoverable flesh and the quality changes which occur during processing and storage as well as the new product development and possibility of its consumer market.

Very little information is available on the suitability of freshwater fish for use in comminuted, gel-type products;

except Zapata (1978) reported that frozen minced sucker flesh showed poor water holding capacity and texture characteristics with low binding, low gelation strength and high losses during cooking. A preliminary test was carried out to predict the gel-forming ability of both fresh and frozen minced sucker flesh, as a basis for frozen fish paste and fish ball formulations, and the quality of their stability under frozen storage.

There are three main objectives in this research project: to determine (1) the feasibility of using suckers, freshwater fish in fish ball formulations and similar types of comminuted fish products, (2) the effect of washing and heat treatment on the shelf-life of minced sucker during frozen storage, and (3) lipid oxidation and product stability. These freshwater fish balls could be a substitute for those prepared from marine fish and be a potential export commodity.

REVIEW OF LITERATURE

Introduction

The quality stability of the fish paste products made from mechanically deboned fish muscle is greatly influenced by the lipid components and the physiochemical conditions of raw fish proteins. It is known that lipid degradation and protein denaturation are associated with undesirable flavor development and textural toughness in the frozen fish products. The formation of the desirable elasticity and rubberiness in the gel-type products is related to both the quality and quantity of myofibrillar proteins that are present in the paste. The present review will discuss the biological background of white suckers, their chemical composition, post-mortem changes, mechanisms of lipid oxidation and lipid-protein polymerization, as well as the quality stability associated with lipid-protein polymerization. In addition, the functional properties of mechanically deboned fish muscle as well as the rheological property of fish paste products will be reviewed in relationship to the textural quality of gel-type products.

The Background of the Sucker

The sucker belongs to the Catotomidae family. It is a fish of rivers and lakes of North America, eastern Siberia, and China. It consists of a large family, mainly North American, of 10 genera and about 65 species. One species Catostomus catostomus occurs in both Asia and North America (Shoemaker, 1942).

The white sucker (Catostomus commersoni) has an average size of one to two pounds. It is entirely a bottom feeder and sucks its food from the bed of the stream or pond. The food consists of the soft forms of animal life such as worms, insect larva, immature mussels, and plant life.

White suckers spawn in the spring, usually from early May to early June. Spawning sites are usually in shallow water with a gravel bottom.

The flesh of the white sucker is edible and under many circumstances highly palatable. It is white, flaky and sweet but bony. It is not, however, highly favored commercially and forms a small part of the commercial catch.

Composition of Fish Muscle

General Composition and Classification

The proximate composition of fish muscle varies widely from species to species (Stansby and Olcott, 1963). The main constituents of edible muscle are as follows: moisture,

28-90%; protein, 6-28%; fat, 0.2-64%, and ash, 0.4-1.5%.

Stansby and Olcott (1963) classified fish into five categories based on their average lipids and protein contents as given in Table 1.

Table 1. Classification of fish according to the fat and protein content.[a]

Classification	Oil Content %	Protein %	Prototype
Low oil-high protein	5	15-20	cod
Medium oil-high protein	5-15	15-20	sockeye salmon
High oil-low protein	15	15	lake trout
Low oil-very high protein	5	20	skipjack tuna
Low oil-low protein	5	15	clams

[a] Stansby and Olcott, 1963.

The protein nitrogen fraction is mainly composed of myofibrillar (59-73%) and sarcoplasmic (20-30%) proteins and 3-10% of stroma or connective tissue (Moorjani et al., 1962). According to Greaser (1979), the protein content of the myofibrils that have been identified are myosin 50±10%, actin 30±10%, tropomyosin 5%, troponin 5%, connectiv 5-7%, C-protein 2%, M-protein 2%, α-actinin 2%, β-actinin <1%,

γ-actinin <1%, I-actinin <1%, filamin <1%, desmin <1%, vimentin <1% and titan <1%.

Average Percentage Yields and Chemical Composition of Mechanically Deboned Sucker Flesh

According to Dawood (1979), the average percentage yield of gutted and deheaded white sucker is 67.60%; and the yield of the mechanically deboned flesh based on % round weight is approximately 47.60%. The chemical composition of mechanically deboned sucker flesh is: moisture 80.70± 0.52%, nitrogen (x 6.25) 16.65±0.02%, fat 2.03±0.15% and non-protein nitrogen 1.70±0.05%.

Variability in the Fatty Acid Pattern of Lipids from Different Batches of Fish of the Same Species

According to Stansby (1981), "In contrast to the generally accepted idea that oils from one species of fish vary only a little, if at all, in fatty acid patterns, there is actually a tremendous variation. Such variation occurs from fish to fish in the same catch, from lots of fish caught in the same general area at different times of the year, from lots of fish caught at the same time but in different geographical location at one date from one year to another. These variations in fatty acid content of oil from the same species of fish is related to the fatty acid content of the feed available to fish from season to season, or from year to year depending upon the fatty acid pattern

in the flesh of these fish."

Stansby (1981) stated that when the oil used in the analysis of fatty acid patterns was prepared from only a few fish, the data from the analytical result are biased, and the only published papers where adequate sample size has been used are related to the commercial fish oil production where often thousands or millions of fish have been used to comprise one sample.

Studies on seasonal variation in the proximate composition of various species of fish have been analyzed with the following results: (1) Skin and head parts are considered to be very much susceptible to the seasonal effects as they undergo the greatest seasonal variation in fat and moisture contents while middle and tail parts show the least variation. Moisture and lipid contents display an inverse linear relationship to each other (Venkataraman et al., 1968; Leu et al., 1981), (2) The season with highest lipid content coincides with the pre-spawning period, while the lean condition of the fish coincides with the spawning period (Venkataraman et al., 1968; Deng et al., 1976; Leu et al., 1981), and (3) Polyunsaturated fatty acids may be responsible for the seasonal variation of unsaturated fatty acid since monoethenoic acid remains rather constant; while the seasonal changes in saturated fatty acid content are due mainly to the variation of palmitic acid content. For fish oil, there are uncontrollable variables due not only to

geographical locations of catch and season of the year, but also to sex, age, diet, etc. (Deng et al., 1976).

Kinsella et al. (1977) determined the fatty acid content and composition of 18 species of freshwater fish. The fat content and composition varied with anatomical location. The anterior ventral regions of trout and salmon contained more lipids than the posterior dorsal sections. There is a marked variation in fatty acid composition among species. Palmitic (C16:0, palmitoleic (C16:1), oleic (C18:1), eicosapentaenoic (C20:5, ω3), and docosahexaenoic (C22:6, ω3) were most abundant fatty acids. Significant quantities of linoleic acid (C18:2 ω6) and arachidonic acid (C20:4 ω6) fatty acids were found in several species. Within one species, the fillets from large fish tended to contain more lipids. Concentration of palmitic acid (C16:0) and oleic acid (C18:1) was significantly ($p < 0.10$) higher in the large fish and arachidonic acid (C20:4) was greater in samples from small fish.

Hayashi and Takagi's (1977) studies on the fatty acid composition of fish affected by excessive stress, indicated that in gilled fish, the ratios of neutral lipids to phospholipids increased with a decrease of the phospholipids. The acid values of the neutral lipids were significantly higher in dead gilled fish. There were minor differences between gilled and impounded fish in the fatty acid compositions of the neutral lipids; while the fatty acid components

in phospholipids of gilled fish showed a remarkable decrease of eicosapentaenoic (C20:5, ω3) and docosohexaenoic (C22:6, ω3) acids. The decrease of polyenoic acids in the phospholipids of the gilled fish might be due to a metabolic peculiarity induced under excessive stress.

Postmorten Changes in Fish Muscle

Degradation of Glycogen

It was assumed that glycogen content is considerably lower in fish muscles than in mammalian muscles. However, the products of postmorten degradation of glycogen are present and undoubtedly contribute to both the flavor and texture of fish (Tarr, 1966).

Lactic acid accumulates in muscle of living fish as a result of exercise or struggling and may also increase after death. The breaking down of glycogen to lactic acid in fish muscles is caused by a sequence of enzymes that occur at amylolytic route, and/or Embden-Meyerhof glycolytic pathway (Macleod et al., 1963).

Certain fish such as tuna may exhibit high muscle lactic acid concentrations at postmortem with corresponding low pH values, which may inhibit bacterial spoilage of fish (Fraser et al., 1961). On the other hand, low pH values cause muscle proteins to approach their isoelectric zones, and consequently they tend to lose their water holding

capacity. This results in a tendency to loss of drip on thawing frozen fish, and causes dehydrated conditions of the fish muscle (Tomlinson and Geiger, 1962).

Hydrolysis of Adenosine Triphosphate (ATP)

According to Tomlinson and Geiger (1962) and Fraser et al. (1961), the ATP content of the rested fish muscles averages about 500 to 800 moles per 100 g of muscle; except in unusual circumstances, ATP is rapidly degraded during post-mortem by a series of enzyme reactions. Kobayashi (1966) indicated that the hydrolysis of ATP to the stage of inosine monophsophate (IMP) is quite rapid, and that the comparative rate of hydrolysis of IMP to inosine is slower. Thus, IMP tends to accumulate in fish muscles shortly after death; and the slow conversion of IMP to inosine that occurs when fish flesh is held in ice is probably an important cause of flavor loss since IMP was found capable of producing a meaty flavor under appropriate conditions.

Inosine, which was produced from dephosphorylation of IMP, is comparatively flavorless, but hypoxanthine, formed by hydrolysis or phospholytic splitting of inosine, is bitter and was considered to be responsible for the bitter taste of the fish that had been held too long in ice after death (Jones, 1963). But, Hashimoto (1965) stated that hypoxanthine does not cause flavor change in fish held in chilled ice until the bacterial count exceeds one million per g of

fish muscle. Therefore, it is necessary to have more research to observe the exact role of hypoxanthine in the flavor of ice-stored fish.

The formation of free ribose in fish muscles was attributed to postmortem degradation of ATP rather than to glucose via postmortem operation of the hexosemonophosphate shunt pathway (Tarr and Leoroux, 1962). Ribose 5-phosphate was shown to be somewhat more reactive than glucose in causing browning (von Tiggerstorm and Tarr, 1965). However, the concentration of pentose phosphates is not as significant as the hexose-phosphates in causing Maillard reactions in heat dehydrated fish flesh.

According to Hahns et al. (1976), nucleotide degradation in fish muscle is an autolytic process involving deamination and dephosphorylation of ATP, resulting in the accumulation of hypoxanthine.

Microbial Degradation

Microbial degradation plays a major role in the rapid deterioration of fresh fish. Microorganisms are present in the surface slime, in the gills and in the intestinal tract of fish (Shewan, 1961), and at death, these invade the tissues. The optimum growth rate of bacteria occurs at temperatures from 10 to 20^{o}C at pH between 6.5 to 7.5 (Haskin, 1961). Elimination of microorganisms on the fish surface by using chlorinated water resulted in delaying

autolytic processes and allowed a doubling of storage life (Kosak and Toledo, 1981).

Deteriorative changes in fresh fish are the result of oxidative deterioration, autolytic reactions, and bacterial growth. Putrefactive odors mainly produced by bacterial metabolites including amines, skatole, indole, hydrogen sulfide, and aldehydes, while excessive growth of bacteria leads to repulsive appearance of spoiled fish (Nair and Lahiry, 1968; Nickelson II et al., 1980).

Microbial counts usually increased during the process of mechanical deboning fish flesh. This is probably due to contamination from processing equipment, and microorganisms are more closely associated with the minced flesh than the intact flesh. The mincing action increases the release of rich nutrients from muscle cells. These nutrients may have contributed to a better environmental condition for the growth of bacteria (Nickelson II et al., 1980).

The bacterial and coliform counts during frozen storage up to 3 months at -25^{o}C, showed slight changes. Frozen minced fish samples were thawed from -25^{o}C to 2^{o}C at different rates; all had the same final bacterial count. Psychrotrophic bacteria in a beef-fish mixture stored at 2^{o}C grow at the same rate as in fish or beef alone (Nickelson II et al., 1980).

Pigment Discoloration

Chen and Chung (1978) studied the factors causing the green discoloration of frozen dolphin fillets. They concluded that green discoloration could possibly be caused by the interaction between a sulfur-containing substance (e.g. cysteine) and ferrous iron from heme, rather than by hydrogen sulfide producing bacteria, e.g. Pseudomonas putrifaciens. They suggested that an effective way to prevent the green discoloration is to assure complete bleeding of common dolphins aboard fishing vessels and to keep the treated fish in utmost fresh condition before freezing. In this case, ice storage is not advisable either aboard ship or on land. During filleting in the processing factory, fillets should be washed thoroughly with chlorinated water containing 5 ppm of available chlorine. Temperature of freezer storage should be kept below -18^{o}C in the center of the fillets.

Fujita and Kanayama (1973) reported that the brown discoloration was found in both frozen fish paste of Alaska pollack and precooked raw material. Brown discoloration was found when Archromobactor brunificans AJ-3230 and Serratia marcescens UFF-115 were inoculated into media containing gluten, glutenin, casein or gelatin. A. brunificans also formed a brown substance on the media containing glucose and histidine or Na-glutamate instead of a protein. But S. marcescens did not form a brown substance in the amino acid media. It is suggested that sufficient heating during

processing can avoid brown discoloration of fish jelly products since brown discoloration occurred most readily under the condition with optimum temperature, sodium chloride concentration, and pH for the growth of these bacteria.

Lipid Oxidation in Food System

Mechanism of Lipid Oxidation

Fish products are more rapidly oxidized and the reactions are more complicated than those of other foods, mainly because fish fats contain more highly unsaturated fatty acids (Ackman, 1967). Unsaturated fatty acids are considered the major initial substrate in lipid oxidation.

The mechanism of lipid oxidation is an autolytic type of reaction, in which the oxidation products themselves catalyze the further reaction and accelerate the rate of reaction as illustrated by Sato and Herring (1973) and Dugan (1976).

1. Initiation

$$RH \xrightarrow{\text{initiator}} R^{\bullet} + H^{\bullet}$$

2. Propagation

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

$$ROOH \longrightarrow RO^{\bullet} + HO^{\bullet}$$

$$RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet}$$

3. Termination

$$R^{\bullet} + R^{\bullet} \longrightarrow RR$$

$$RO^{\bullet} + RO^{\bullet} \longrightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$$

where RH = unsaturated fatty acid in which H is labile to be attacked by either an initiator or free radical, on a carbon atom adjacent to a double bond.

$R^{\bullet}$ = free radical formed by removal of a labile hydrogen.

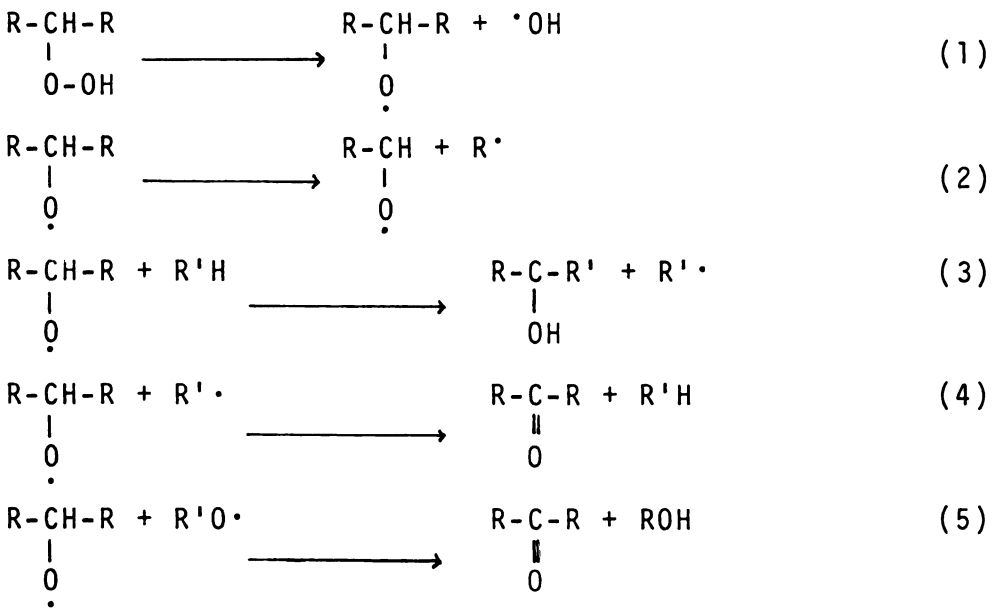
$ROO^{\bullet}$ = oxidized lipid radical or peroxy radical.

Initiation step involves the formation of a free radical species. The initiation of lipid free radical in fish products could be attributed to heat, light, ionizing radiation, enzymes, heme substances and free radicals from any source (Lea, 1962; Olcott, 1962).

The absorption of oxygen by the minced tissue of fresh fish is both enzymatic and nonenzymatic in nature (Olcott, 1962). According to Sato and Herring (1973), the free radicals can combine with atmospheric oxygen to form peroxide free radicals, which can then attack the intact lipid to form more free radicals that further propagate the chain reaction. Finally, the reaction that occurs in between radical species is terminated by formation of non-free radical products.

In autoxidation of lipids, the unsaturated fatty acids are oxidized to conjugated hydroperoxides which are called primary products of oxidation. These hydroperoxide are further decomposed to form secondary products (Keeney, 1962).

Keeney (1962) suggested that the hydroperoxide decomposition followed the same mechanism as in the free radical chain reaction that occurred in autoxidation:



Keeney (1962) indicated that in reaction (1), the hydroperoxide is cleaved to alkoxy and hydroxyl free radicals; reactions (2) to (5) illustrate the reaction of alkoxy free radical with other free radicals or unoxidized fat molecules to form secondary products which include carbonyl compounds, alcohols, semi-aldehydes, acids, hydrocarbons, lactones, epoxides and esters. These secondary products

are responsible for the flavor or odor of the oxidized lipid food system.

Role of Singlet Oxygen in the Lipid Oxidation

The presence of hydroperoxides with nonconjugated double bonds among the primary reaction products indicated that singlet oxygen was the reactive intermediate in photosensitized reactions which is differred from free radical initiated autoxidation reactions (Vianni, 1980). Rawls and van Santen (1970) proposed the following mechanism by which the singlet oxygen could be formed by photoxidation in the presence of a sensitizer, and presented evidence that singlet oxygen reacts with lipids at a rate which is 1,450 times faster than triplet oxygen:

$$S + h\nu \longrightarrow {}^1S^* \longrightarrow {}^3S$$

$${}^3S^* + {}^3O_2 \longrightarrow {}^1O_2 + {}^1S$$

$${}^1O_2 + RH \longrightarrow ROOH$$

$$ROOH \longrightarrow \text{free radicals}$$

where

${}^1S$ = singlet sensitizer

${}^1S^*$ = excited singlet state sensitizer

${}^3S^*$ = excited triplet state sensitizer

${}^3O_2$ = ground state triplet state oxygen

${}^1O_2$ = excited singlet state oxygen

Plant and animal tissues consist of photosensitive compounds such as chlorophyll, pheophytin, and myoglobin which are required to convert the triplet oxygen to its singlet state, in order to promote the photoxidation reaction in a food system.

Aurand et al. (1977) reported that singlet oxygen was the immediate source of the hydroperoxides that initiated milk lipid oxidation which was catalyzed by light, copper and xanthine oxidase. In light-induced oxidation, riboflavin acted as sensitizer by producing singlet oxygen directly from its photosensitized triplet state; while in copper and xanthine oxidase systems, singlet oxygen was formed by dismutation of the superoxide anion ($O_2^-\cdot$) which are formed in these systems. Oxidation was prevented in all three systems by using a singlet oxygen trapper or quencher. Lipid oxidation could be prevented in systems containing copper and enzymes by inclusion of superoxide dismutation enzymes which prevent the formation of singlet oxygen from superoxide. Thus, singlet oxygen is the initial reactant with lipid when oxidized in systems that are not induced by light. However, there is evidence that singlet oxygen does not participate in the oxidation of lipids that contain no sensitizers (Cort, 1974).

The photosensitized oxidation was inhibited by β-carotene, a singlet oxygen quencher, but was not inhibited by butyl hydroxytoluene (a free radical stopper). Thus,

the singlet oxygen oxidation differs from air oxidation.

Factors Affecting Lipid Oxidation

Lipid composition and structure. The hydrogen liability of the methylene carbons on which the free radicals are formed can be grouped according to the number and type of unsaturated bonds in the fatty acid molecules (Sattar and Deman, 1976). However, in a more complex food system, the oxidation of foods does not necessarily coincide with the degree and type of unsaturated lipids in foods. Raghuveer and Hammond (1967) found that the position of the unsaturated fatty acid in the triglyceride could influence the oxidation rate of the lipids. The rate of lipid oxidation would be slower if more of the unsaturated fatty acids were located in position 2 of triglycerides than if they were located in the 1- and 3- position. According to Brockerhoff et al. (1968), the distribution of fish fatty acids in a triglyceride molecule are as follows: position 1 attracts saturated and monounsaturated acids; while position 2 is filled with polyunsaturated and short chain acids; all fish accumulate C16:0 at 2-position except the trout; and position 3 contains mainly long chain fatty acids.

Oxidation catalysts. According to Ingold (1962) heavy metals, mainly those having two valency states with oxidation-reduction potentials, increase the rate of lipid oxidation. These heavy metals may act as secondary catalysts

of oxidation where they serve as electron donors to hydroperoxides to produce oxy-free radicals (RO·). The M^{+3} can be reduced back to M^{+2} via reaction with hydroperoxides to form hydroperoxy radicals (ROO·).

$$ROOH + M^{+2} \longrightarrow RO\cdot + M^{+3} + \cdot OH$$
$$ROOH + M^{+3} \longrightarrow ROO\cdot + M^{+2} + H\cdot$$

These two reactions then start another chain sequence in the oxidizing lipids. Green and Price (1975) stated that heme proteins can catalyze lipid oxidation, but which state of heme iron is responsible for catalyzing lipid oxidation is disputed. Younathan and Watts (1960) and Green (1969) believe that the oxidized form (Fe^{+3}) is responsible for catalyzing oxidation in lipid; while Brown et al. (1963) and Hirano and Olcott (1971) indicate that both Fe^{+2} and Fe^{+3} are equally active as catalysts.

Antioxidants. According to Labuza (1971), phenolic type compounds such as butylated hydroxy anisole (BHA), butylated hydroxy toluen (BHT), and tocopherol can act as free radical stoppers by donating hydrogen to the free radicals; chelating agents such as ethylenediaminetetra-acetic acid, citric acid and polyphosphates are free-radical-production preventors which tie up metal catalysts. Ascorbic acid has a synergistic effect when used with phenolic type antioxidants because ascorbic acid can donate hydrogen to a phenoxy radical or function as an oxygen

scavenger in some systems. Thus, ascorbic acid plays a vital role in terminating the free radical formation.

Lipid Oxidation in Fish During Iced and Frozen Storage

Most fish fat contains more than 50% unsaturated fatty acids, of which about half are polyunsaturated (Ackman, 1976). Lipid oxidation is one of the major factors contributing to difficulties in the preservation of fish quality during frozen storage, especially in the fatty fish (Ke and Ackman, 1976).

According to Licciardello et al. (1980), hakes contain a layer of red muscle just beneath the skin that is rich in highly unsaturated fats and hematin pigments, which makes the flesh prone to the development of oxidative rancidity. The red muscle has higher fat content than white muscle. Therefore, when the fillet is skinned, it exposes the fatty layer to the air and the flesh becomes more susceptible to oxidative rancidity, also accompanied by the development of gray or brown discoloration probably due to formation of metmyoglobin.

Hydrolytic cleavage of lipids during frozen storage of fish is an important factor contributing to the quality deterioration of the products (Nair et al., 1976). Bosund and Ganrot (1969a) reported that storage of Baltic herring for up to 12 weeks at -15^{o}C resulted in an increase of free

fatty acid content in both dark and white muscles. The increase was due primarily to hydrolysis of lecithin, cephalin and to a lesser extent to triglycerides, roughly 45% and 75% of the free fatty acid formed in the dark and white muscle respectively was a result of phospholipid hydrolysis. The remainder was of triglyceride hydrolysis. The hydrolysis of lecithin was faster than that of cephalin. Nair et al. (1976) and Mai and Kinsella (1979a) agree that free fatty acid production in frozen fish was mainly associated with the hydrolysis of phospholipids. There were no significant changes in triglycerides and unsaponifiable matter during frozen storage at -18°C.

The occurrence of rancidity during ice storage in raw fresh fish has not been considered a serious problem since deterioration by microbial action usually takes place before chemical changes are significant (Olcott, 1962). However, oxidative rancidity was evident in mullet and bluefish following 3 to 5 days of ice storage. Their total plate counts were low, but TBA values increased rapidly near the 4th day of ice storage. Fisher and Deng (1977) indicated that heme iron was the major catalyst of lipid oxidation in mullet flesh.

A major cause of quality loss in frozen fish is lipid oxidation, which not only affects flavor but also causes undesirable textural changes through an interaction of protein and lipid oxidation products. The unsaturated fatty

acids (C20:5, C22:6, C18:1) have been found to decrease during frozen storage at -20°C for one month (Takama, 1974). McGill et al. (1974, 1979) used gas chromatography-mass spectrometry to identify the major compound that causes the off-odor of frozen stored fish. They confirmed that the n-3 polyenoic acids present in cod lipids produced hept-cis-4-enal which was responsible for the cold storage odor.

Ke et al. (1978) stated that the formation of free fatty acids by lipid hydrolysis in both skin and muscle of frozen mackerel fillet was increased significantly by microwave preheating for various radiation periods. This is probably due to the microwave energy disrupting muscle membranes and liberating the normal enzymes and their substrates. The possible role of catalysis in the initiation of lipid oxidation by microwave heating is not fully understood. But, the microwave at 2,450 MHz used for pretreatment of the fish is great enough to activate oxygen on either direct attack basis, or to promote the transition state of oxygen from its low energy triplet state to the high energy singlet state, using tissue pigments (Rawls and van Santen, 1970) and xanthine oxidase (Aurand et al., 1977) as sensitizers. Hanaoka and Toyomizu (1979) studied the enzymic decomposition of phosphatidylcholine (PC) in carp muscle during frozen storage at 0, -3, -5, -7, -11°C for 10 days. They concluded that PC value was highest at -5°C and lowest at 0°C. Rapid freezing of minced carp

muscle has higher PC value than those of slow freezing. They suggested that the degradation accompanied by ice formation resulting from freezing played an important role in the acceleration of PC decomposition in frozen muscle.

Toyomizu and Hanaoka (1980a,b) reported that during the storage of minced fish muscles at -5°C for four weeks, lipid oxidation proceeded in Pacific mackerel and sardine, fatty fish, but hardly proceeded in plaice, carp and lean fish and TBA values exhibited close linear relationship to lipid oxidation of the Pacific mackeral during frozen storage ($r = 0.96$). Susceptibility to lipid oxidation of the Pacific mackerel during frozen storage at -5°C proceeded in the following order: skin > viscera > dark muscle > white muscle. While in the round, the same order of the lipid oxidation was observed, but was markedly less pronounced than that of the minced except the skin. The preferential lipid oxidation in the skin was attributed to the property of skin lipids being liable to autoxidation. They suggested that a tendency toward lipid oxidation in both lean and fatty fish could be predicted in advance of the storage by measuring TBA values of fresh skin after having incubated its homogenate for 2 hours.

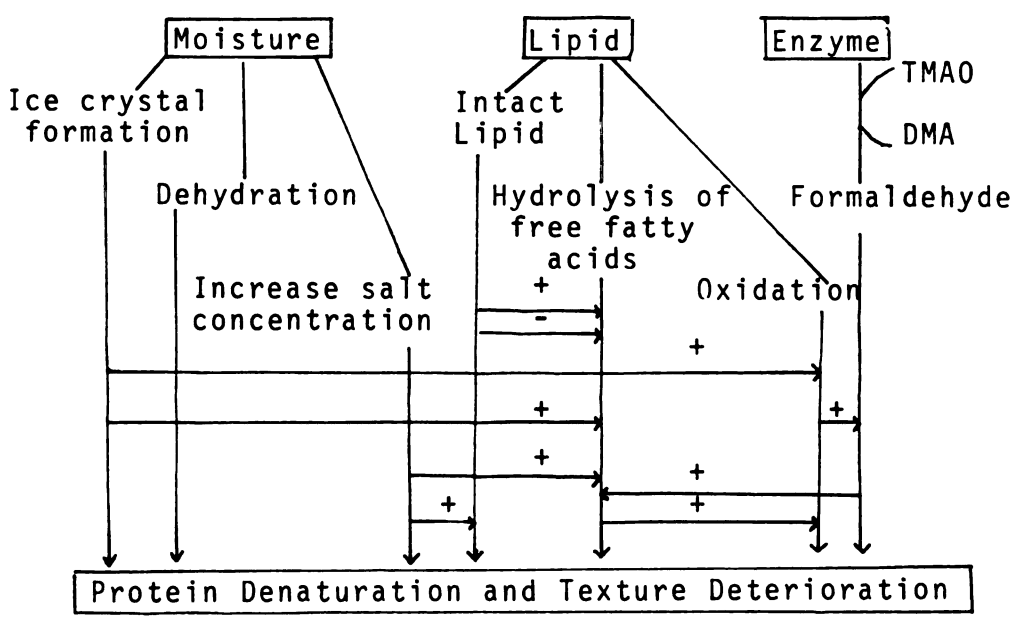
Lee and Toledo (1977) reported that the lateral tissue (red muscle) along the visceral cavity and bone marrow exudate appeared to be most susceptible to oxidative rancidity. Rate of TBA value change was very rapid when these

muscles had contacted with iron surfaces. Silberstein and Lillard (1978) studied the perooxidative effect of hemoglobin, myoglobin, and total heme and nonheme pigments in mechanically deboned mullet. They indicated that myoglobin has greater catalytic effect than hemoglobin. The rate of lipid oxidation increased as the ratio of hemoglobin to myoglobin decreased, i.e. oxidative influence of hemeprotein on the minced fish was accelerated as the concentration of myoglobin increased.

Protein Denaturation and Texture Deterioration

Interaction between moisture, lipids and enzyme on protein denaturation and texture deterioration

Shenouda (1980) suggested a diagram that illustrated the factors which affect directly or indirectly fish protein denaturation during frozen storage as follows:



Shenouda stated that change of the state and concentration of moisture, lipid stability, and enzyme activity could influence the muscle protein denaturation directly or indirectly through their effect on each other; as it is indicated in the above diagram, the horizontal arrow represents the indirect factors and the vertical pathways represent the direct factors. The occurrence and accumulation of one of these factors could trigger or retard the reaction rate of others. For instance, high salt concentration resulting from protein denaturation could stimulate the hydrolysis of lipids and accelerate the liberation of free fatty acids.

Freezing brings about crystallization of water present in the fish muscles (Sikorski et al., 1976). The distribution and size of ice crystals depend upon the condition of the muscle, the rate of freezing, the storage time, and temperature fluctuations (Love, 1968). In pre-rigor fish, the crystallization of ice takes place intracellularly regardless of the rate of freezing. On the other hand, in post-rigor fish, if frozen slowly, ice crystals form in the extracellular fluids and grow at an expanse of water which diffuses out of the cells; while at faster freezing rates, a large number of tiny ice crystals grow both within and outside of the cells. As a consequence, the decreased amount of liquid water available to the proteins causes the increase in concentration of tissue salts. The

mechanical damage to the muscle structures due to the growing of ice crystals (Love, 1968; Kent, 1975) resulted from fluctuations in freezing temperatures (Dyer and Dingle, 1961).

According to Castell et al. (1970) and Sikorski et al. (1976), inorganic salts affect protein denaturation in frozen fish most probably by depressing the freezing point of the tissue fluids, causing dehydration, influencing the interfacial tension, and by ionic interaction with charged groups of the polypeptide side chains. Inorganic salts can interfere with the conformation of protein by participating in the formation of lipid-protein complexes, reacting with nitrogen and oxygen functional groups of proteins, and inducing lipid oxidation, which in turn, may bring about protein insolubilization. Pigott and Shenouda (1975) reported that the presence of Ca^{++} as well as a solution with high ionic strength favors polymerization of actin and binding of both neutral and polar lipids. Calcium ions enhance the polymerization of G-actin to F-actin and by so doing favor protein-lipid interaction. Buttkus (1971) added Cu^{++} ions to the homogenate of trout myosin, causing a rapid loss of -SH groups and aggregation of the myosin molecules.

Many salts exhibit a solubility effect at low ionic strength (0.5-1μ) by associating with ionic linkages of the protein, rupturing the bonds, and enforcing hydration of

the newly built associations (Lewin, 1974). At higher salt concentration, the competition of inorganic salts for water may result in a salting-out effect, whereby the number of hydrophilic groups associated with water that buttress the solubility of the macromolecule can be reduced, and decrease the amount of water for protein. In addition, at higher salt concentrations (above 1M), most inorganic salts cause a proportional increase in the surface tension of the solutions, resulting in an enhancement of hydrophobic interactions.

Effect of Cryoprotective Agents on Prevention of Protein Denaturation

Noguchi et al. (1975a,b,c; 1976) have done intensive studies on the control of denaturation of fish muscle proteins during frozen storage by using cryoprotectants. They determined the cryoprotective effect of different compounds such as amino acids, carboxylic acids, carbohydrates and their derivatives; and they found that the following compounds exhibited better cryoprotective effects among those tested: (1) glutamate, aspartate, proline, cysteine, glutamylcysteinylglycine, acetylglutamate; (2) malonic, maleic, malic, lactic, tartaric, gluconic, glycolic, methylmalonic and glutaric acid; and (3) glucose, fructose, sucrose, lactose, stachyose, melezitose, glycerol, ethylene, glycol, sorbitol, β-glycerolphosphate and glucose-1-phosphate.

These researchers proposed that the effective cryoprotective agents for fish muscle should have the following properties: (1) the molecule must possess an essential group of either -COOH, -OH, or $-OPO_3H_2$, and more than one supplementary group of the type -COOH, -OH, $-NH_2$, -SH, $-SO_3H$, or $-OPO_3H_2$; (2) the functional group of the compound must be suitably spaced and properly oriented to the protein molecules of the fish muscle; and (3) the molecules must be comparatively small.

Matsumoto (1980) suggested that cryoprotectants acted as water structure modifiers by binding with protein molecules and preventing the unfolding of globular proteins. This effect was due to increased hydration of the protein molecules and an increased resistance against displacement of water even when the system was frozen.

Complex Character of Protein Deterioration in the Presence of Hydroperoxidizing Lipid in Frozen Fish

The result of lipid hydrolysis is detrimental to quality in lean fish, which at higher concentrations of neutral lipids is dispersed in the muscle tissue in the form of droplets; their competition for hydrophobic binding sites may reduce the participation of polypeptide side chains in hydrophobic interaction with fatty acids hydrocarbon residues. Lipid autoxidation which results in the formation of free radicals and reactive scission products

enhance protein changes regardless of the concentration of neutral lipids in the muscle cells. Fish containing phospholipids with a large proportion of polyunsaturated fatty acids, which are most susceptible to autoxidation, should be especially liable to protein deterioration due to these effects (Sikorski et al., 1976).

Dyer and Dingle (1961) reported that formation of free fatty acids preceded the loss of extractability of myofibrillar proteins and that the latter was more rapid in lean species. Ohta and Nishimoto (1964) demonstrated that addition of fatty acids to minced mackerel flesh decreased the extractability of proteins from samples stored at -10 and -20^{o}C at pH 6.4 to 6.8. This is because the interaction of free fatty acids with myofibrillar proteins produces a network of cross-links which increases the resistance of the muscle fibers of fragmentation and reduces the protein solubility.

Fatty acids liberated from phospholipids as a result of hydrolysis,may attach themselves to appropriate binding sites of either neutral lipid droplets or hydrophobic, polar, or ionized fragments of the peptide chains (Sikorski et al., 1976). When they interact with neutral lipids, they become inactive; when they bind to polypeptide side chains of polar groups, they may decrease the protein solubility due to formation of intermolecular hydrophobic-hydrophilic or hydrophobic-ionic linkages, especially at

appropriate concentrations of inorganic ions (Pigott and Shenouda, 1975).

Oxidized lipids in the lipid-protein system are known to induce polymerization and aggregation of the proteins, resulting in decreased solubility and formation of color complexes (Karel, 1973). According to Castell (1971), lean fish muscle contains 0.5 to 1.0% unsaturated lipids, but during frozen storage it rarely goes rancid, as indicated by thiobarbituric acid values or rancid odor. The lipids oxidize, but instead of forming carbonyls and other compounds associated with rancidity, they become bound in lipid-protein complexes which account for the toughened texture of poorly stored frozen fish.

Funes and Karel (1981) stated that the dominant mechanish of protein polymerization after exposure to peroxidizing linoleic acid is the transfer of free radical from lipid to protein, and subsequent free radical polymerization. Roubal and Tappel (1966) proposed a free radical displacement reaction as follows:

$$P\cdot + P \longrightarrow P\text{-}P\cdot$$

$$P\text{-}P\cdot + P \longrightarrow P\text{-}P\text{-}P\cdot$$

where P = protein

The concentration of protein radicals is higher with an increase in lipid oxidation and a decrease of water activity (A_w). Extensive protein-protein cross-linking occurs in a food system at A_w values between 0.75 and 0.40, and

significantly less at lower water activity. Schaich and Karel (1975) postulated that high water activity may reduce the concentration of radicals by favoring their cross-linking. The availability of water may control the rate of cross-linking via terminating the radical formation by donation of hydrogen atoms and/or by colligating two protein radicals together as follows:

$$P^{\bullet} + P^{\bullet} \longrightarrow P\text{-}P$$

Gardner (1979) summarized the following statement in his review of lipid hydroperoxide reactivity with proteins and amino acid: (1) production of lipid hydroperoxides in an inadequately processed foods is catalyzed by lipoxygenase; (2) the hydroperoxides and their scission products are potentially reactive compounds that bring about the degradation of food proteins and amino acids; (3) formation of lipid-protein complexes is resulted from the exposure of protein to peroxide lipids; (4) the degradation of food quality resulted from the interaction between lipid hydroperoxide and proteins are characterized by protein-protein links, protein scission, protein-lipid aducts and amino acid damage; (5) formation of covalent bonds between protein and the secondary products of the lipid oxidation is another detrimental factor that reduce the product stability.

Karel et al. (1975) proposed a mechanism to illustrate the formation of lipid-protein polymers as follows:

$$LH + R^{\bullet} \xrightarrow{O_2} L^{\bullet} + LOO^{\bullet} + LOOH + RH + S$$

$$PH + L^{\bullet} + LOO^{\bullet} \longrightarrow P^{\bullet} + LH + LOOH + POO^{\bullet}$$

$$P^{\bullet} + P^{\bullet} \longrightarrow PP$$

$$P^{\bullet} + POO^{\bullet} \longrightarrow POOP$$

$$P^{\bullet} + L^{\bullet} \longrightarrow PL$$

$$POO^{\bullet} + L^{\bullet} \longrightarrow POOL$$

$$POO^{\bullet} + LH \longrightarrow POOL + H^{\bullet} + POOH + L^{\bullet}$$

$$PH + S \longrightarrow RHS + PHSPH$$

where:

LH = lipid

PH = protein

$R^{\bullet}$ = free radical

$L^{\bullet}$ = lipid free radical

$P^{\bullet}$ = protein free radical

$LOO^{\bullet}$ = lipid peroxy free radical

$POO^{\bullet}$ = protein free radical

S = lipid scission product

Various aldehydes are the scission products of lipid hydroperoxides; they act covalently with proteins that contribute both flavor and color to a specific food system (Eskin et al., 1977). Non-enzymatic browning results from interaction between peroxidizing lipids and proteins. For example, the highly unsaturated nature of fish lipids results in the browning of fish. The aldehydes resulting

from lipid oxidation are usually volatile and often emanate potent odors. In browning reactions, these aldehydes produce gluey odors and fishy aromas (Gardner, 1979).

In food systems with low water activity, the protein scission is more likely to occur than protein-protein cross-linking when peroxidized lipid is mixed with protein. At the low water activity state, protein peroxides were formed through oxygen attack on α-carbon-centered radicals (Gardner, 1979). Subsequent cleavage at the peroxide-bearing α-carbon resulted in protein scission and an increased amide content. Amino acid residues in protein are damaged from exposure to lipid hydroperoxide regardless of whether the mixture of peroxidized lipid and protein is incubated in an aqueous system or dehydrated state (Zirlin and Karel, 1969).

Histidine, cystine/cysteine, methionine, lysine and tyrosine are the amino acid residues that are most sensitive to damage by lipid hydroperoxides. The formation of thiol radical from cysteine is facile. The oxidation of methionine sulfoxide is probably due to the ease of delocalization of electrons of sulfur. Products from the nonsulfur amino acids can be explained on the basis of radical formation at the α-carbon and to some extent on the side chains (Tannenbaum et al., 1969).

Young and Karel (1978) postulated that the degradation of histidine involves the following sequence: (1) formation

of an α-carbon radical by deamination; (2) hydroperoxidation of the α-carbon, and (3) hydroperoxide hydrolysis which led to imidazole lactic acid and imidazole acetic acid. Formation of lysine products was due to the radical attack at the α-carbon, and side chain carbons. Among the side-chain carbon the ε-carbon was the most labile. Schiff base formation is a particularly important degradation reaction for lysine (Gardner, 1979).

Enzymatic Activity of Trimethylamine Oxidase As Related to Protein Denaturation During Frozen Storage

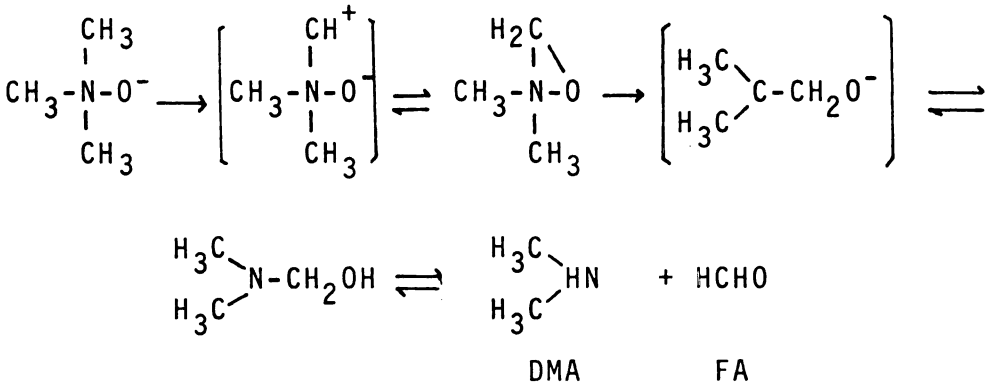
Distribution of trimethylamine oxide (TMAO) and trimethylamine oxidase (TMAOase)

TMAO plays an important role in maintaining nitrogen balance in the marine fish. The magnitude of the natural occurrence of TMAO in marine fish differs in various species; on the other hand, TMAO is scarcely found in freshwater fish. Both elasmobranchs (cartilage fish) and mollusks (squid) contain a higher amount of TMAO than the teleosts (bony fish). Among the teleosts, the gadoid family, such as cod, pollack, haddock, whiting and hake contain the highest amounts of TMAO; whereas the flatfish have the lowest amount; crustaceans, such as shrimp and crab, contain moderate amounts of TMAO; the bivalves (e.g.

clams, oysters) and echinoderms (e.g. starfish) contain very low levels of TMAO (Konosu et al., 1974; Suyama and Suzuki, 1975).

Disruption of fish muscle fibers during the mechanical deboning process leads to breakdown of TMAO to DMA and formaldehyde (FA) by the action of TMAOase. The distribution of TMAOase was detected by measuring its end products DMA and formaldehyde (Shenouda, 1980).

The mechanism of formaldehyde and DMA formation was postulated by Harada (1975) as follows:



According to Harada (1975) and Hiltz et al. (1976), TMAOase exists only in a limited number of marine animals, and its activity varies widely among species, types of tissue, and storage temperatures. Fish, squid, bivalves, and gastropodes show some capacity to form DMA and formaldehyde; while scallops, lobsters and shrimp were found lacking in TMAOase (Castell et al., 1970). Godoid family was found to have the highest TMAOase activity; in which the formation

of DMA and formaldehyde was greatest in species that had large amount of dark lateral muscle in fillets.

Harada (1975) reported that TMAOase is relatively heat-stable; its activity was maintained at temperatures up to 60°C for 5 minutes. Lall et al. (1974) noted that heating the silver hake fillets or minced flesh to an internal temperature up to 60°C were not effective in inactivating the enzyme during subsequent frozen storage at -10°C, but when the internal temperature reached 80°C, the preheating treatment was highly effective in arresting the enzyme action.

Effect of the Formation and Accumulation of Formaldehyde in Fish Muscle

Formaldehyde is a very reactive compound capable of interacting with amino, amido, thiol, guanido, phenolic, imidazole, indolyl residues (Sikorski et al., 1976). Therefore, extensive accumulation of formaldehyde in frozen minced fish is often accompanied by loss of extractability of myofibrillar proteins. The rate of formaldehyde accumulation is higher in dark muscle, intestine, kidney, blood, liver, and unwashed minced flesh than that of white muscle, washed muscle, and intact fillets (Babbit et al., 1974; Dingle and Hines, 1975).

Ishikawa et al. (1978a,b) pointed out that TMAO could act as a peroxide decomposer and, at the same time, show a

synergistic effect on the activity of γ-tocopherols in inhibiting the lipid oxidation. Thus, the depletion of TMAO concentration and formation of formaldehyde via the catalyzing reaction of TMAOase, would result in accelerating the hydrolytic decomposition of the fish lipids, especially the triglycerides, phospholipids, and sterol esters (Ostyakova and Kosvina, 1975). According to Childs (1973), sensory evaluation revealed that tissue containing formaldehyde became tougher and increased in its water-holding ability, but lacked juiciness. The texture was evaluated as being rubbery and spongy.

Processing of Fish by Mechanically Deboning Machine

The principle of the mechanical deboning machine involves squeezing the cleaned, gutted fish through a honeycomb of narrow orifices. This action results in the extrusion of the softer texture flesh, leaving behind most of the tougher skin, bones and scales as a wasted residue (Wong et al., 1978).

The size of the orifices can affect the amounts of bone residues left along with the minced flesh. There are large number of fine, long bones recovered from minced herring flesh obtained through the use of drums with 5- and 7- mm openings, while the 2- and 3- mm perforated drums yielded minced flesh containing little or no scale fragment, and extremely short bones in minced pollock, rockfish and

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herring (Wong et al., 1978). The variability of the bone residue present in minced fish appeared to be a function of processing methods and raw material, but not of species (Patashnik et al., 1974).

The yield of flesh recoverable from mechanically deboned fish of different species ranged from 37 to 60%, in comparison to the fillet yields on the same species which ranged from 25 to 30% (Miyauchi and Steinberg, 1970). Rippen (1981) stated that the yield of mechanically deboned fish flesh depends on fish species and machine operation. The average yield of minced white sucker flesh is approximately 50% of the round fish, containing 0.13% bone residues (Zapata, 1978).

Su et al. (1981a,b) stated that the presence of trace amounts of fish skin and internal organs, particularly liver, kidney and visceral tissue is responsible for increasing the alkaline protease levels in minced fish flesh. They indicated that alkaline protease activity in croaker kidney and liver is several thousand fold higher than in skeletal muscle. Addition of kidney and liver tissue to mechanically minced fish flesh that had been thoroughly washed, resulted in increased protease activity and degradation of fish tissue upon comminuting and cooking at 60^{o}C. Failure to thoroughly wash eviscerated fish prior to mincing appears to result in the retention of tissue from intestinal organ.

Okada et al. (1973) and Miyauchi et al. (1975) also reported that presence of blood pigments, particles of skin, membrane from peritoneal cavity, scales and bone can contribute to the deterioration of fish flesh during subsequent storage. Contact of fish flesh with iron parts of a mechanical deboner can also accelerate oxidative rancidity and discoloration (Lee and Toledo, 1976).

The mechanical deboning process not only breaks down the myofibrils at Z and M bands but alters the composition of fish muscle. Significant amounts of lipid and heme components are released from the bone marrow and subsequently accumulated in the minced flesh (Schnell et al., 1973). A higher content of dark muscle was obtained in the minced muscle in the fillet. The former accounts for a higher hemoglobin content (Froning, 1981).

Functional Properties of Fish Muscle Proteins

Functional Classification of Muscle Proteins

According to Goll et al. (1974), the functional role of muscle proteins can be classified into 3 groups on the basis of their solubility: (1) the myofibrillar proteins are soluble in a salt solution with ionic strength greater than 0.30, (2) sarcoplasmic proteins are soluble at ionic strength less than 0.05, and (3) the stroma proteins or connective tissue proteins which are insoluble in neutral

solutions.

Briskey and Fukazawa (1971) and Goll et al. (1974) stated that myofibrillar proteins are responsible for the textural qualities of the comminuted fish and meat products, namely for the water binding capacity, gel forming ability, and emulsifying ability. In contrast, the sarcoplasmic proteins of fish muscle were considered to be responsible for the formation of undesirable flavor, color and protein denaturation due to postmortem enzyme activities (Bai and Radola, 1977).

Water-holding Capacity

The ability of processed meat to hold intrinsic and added water is an important factor in determining quality and product acceptability (Dawood, 1979). The rheological behavior of comminuted muscle products could be affected by the time of postmortem, pH, the ratio of myofibrillar to sarcoplasmic proteins, added ingredients such as sodium chloride, polyphosphate, starch, protein substitutes, etc. (Hamm, 1975; Lee and Toledo, 1979).

Miller et al. (1968) reported that pH affected water retention and swelling of meat products. The loss of moisture by meat increased as its pH approached the isoelectric point of its protein at pH 5.5. The water holding capacity of meat increased as the pH was either decreased or increased away from the isoelectric point (Hamm and

Deatherage, 1960). Freshly slaughtered meat gradually decreased in pH with the onset of rigor mortis. Water holding capacity reached a minimum when rigor mortis was complete. Aging the meat restored water binding ability, but never to the original levels of the living tissue. Treating meat with polyphosphate in the presence of the proper types and levels of alkaline-metal ions and optimum ionic strength and pH, restored its original water holding capacity (Ellinger, 1972).

According to Hamm (1975), the retention of high water holding capacity could be due to binding of chloride ions from the added sodium chloride by the muscle proteins. This is true when ATP is still present in the muscle tissue before on-set of rigor mortis. Hamm postulated that the combined action of the salt and ATP caused the peptide chain to unfold, leaving such large distances between the chains that the divalent cations released by breakdown of ATP were unable to cross-link the chains. Thus, water was able to reach the numerous hydrogen bonding sites necessary for complete hydration of the proteins.

The ATP present in pre-rigor muscle as well as the inorganic polyphosphates used in the fish industry to treat fillets before freezing for the purpose of reducing drip after thawing are capable of complexing divalent cations which inhibit the hydration of myofibrillar proteins, thereby increasing water holding capacity and reducing

thawing-drip loss (Love and Abel, 1966).

Ellinger (1972) found that sodium polyphosphates were highly effective in increasing the water holding capacity of the Japanese kamaboko, a steamed fish paste, by aiding in formation of protein gels which were important to its formation.

Seafood, both fin fish and shell fish, can benefit from phosphate treatment. Seafood readily loses large amounts of its fluid content. Treatment with phosphates can retain its natural moisture content and remain more succulent (FMC Food Chemical Codex,1980).

Binding Properties and Gel Forming Ability

The binding property of fish muscle is attributed to the myofibrillar proteins and degree of hydration (Sato and Nakayama, 1970). Native tropomyosin and actin promoted the binding capacity of meats when pyrophosphates were incorporated into the sausage system; the promotion of binding effect was due to the interaction between myosin and F-actin which resulted in increasing the viscosity of the meat protein (Nakayama and Sato, 1971a). The binding property of heated actomyosin gel increased with increasing myosin concentration (Nakayama and Sato, 1971b). It also increased when F-actin was present in an optimum ratio of myosin, and a greater binding effect was found when tropomyosin was present (Nakayama and Sato, 1971c).

Webb et al. (1976) reported that mechanically deboned tissue had higher amounts of sarcoplasmic protein content as compared with the hand-deboned tissue; stroma protein was somewhat higher in hand-deboned tissue than in mechanically deboned tissue. Nishimoto and Koreeda (1979) indicated that the washed minced fish muscle contained higher actomyosin to sarcoplasmic protein ratio which resulted in a greater gel-forming capacity. As a consequence, the insoluble sol formed by sodium chloride extraction, from mechanically deboned fish muscle may have resulted in a less firmer texture since the mechanically deboned muscle had a lower ratio of actomyosin/sarcoplasmic protein as compared with hand-deboned tissue.

Itoh et al. (1980a) found that the solubility of actomyosin decreased and the molecular weight of protein molecules increased during the gel formation. These changes are impaired to some extent by adding the SH reagents to actomyosin, indicating that SH groups are involved in the changes to higher molecular weight protein molecules during the gel formation. Itoh et al. (1980b) further suggested that the formation of the polymeric molecules of protein resulted from the formation of intermolecular -S-S bonds in the heated actomyosin gel.

Rheological Property and Structure of Kamaboko

Yutaka et al. (1981) studied the species variations in the gel forming characteristics of fish meat paste. They

concluded that two reactions occurred in the gel-forming processes: (1) Suwari, a structure-setting reaction proceeding at temperatures below 50°C, promoted specially at 30-40°C, and (2) modori, a structure-disintegrating reaction occurring at temperatures between 50 and 70°C, optimum at 60°C. Their results agree with Cheng et al. (1979) findings Based on these two phenomena the gel-forming characteristics of fish meat paste were summarized into the following 4 categories: (1) difficult-setting and difficult-disintegrating group, e.g. meat from shark, chicken and rabbit, etc., (2) difficult-setting and easy-disintegrating groups, e.g. the red meat fish, (3) easy-setting and easy-disintegrating group, e.g. sardines, croaker and cold water fish, and (4) easy-setting and difficult-disintegrating group.

Takagi (1973a) studied the viscoelastic network structure of kamaboko. He indicated that the kamaboko forming ability of brayed fish meat decreases with higher degree of modori, since modori of the brayed meat lowers the intensity of the entanglement between chain segments in kamaboko. Kamaboko prepared from undenatured brayed meat formed a cross-linked gel, while kamaboko prepared from brayed meat that has undergone a higher degree of modori resulted in a non-crosslinked gel. The average molecular weight of the network chain molecules in kamaboko decreased with the degree of progressing modori phenomenon.

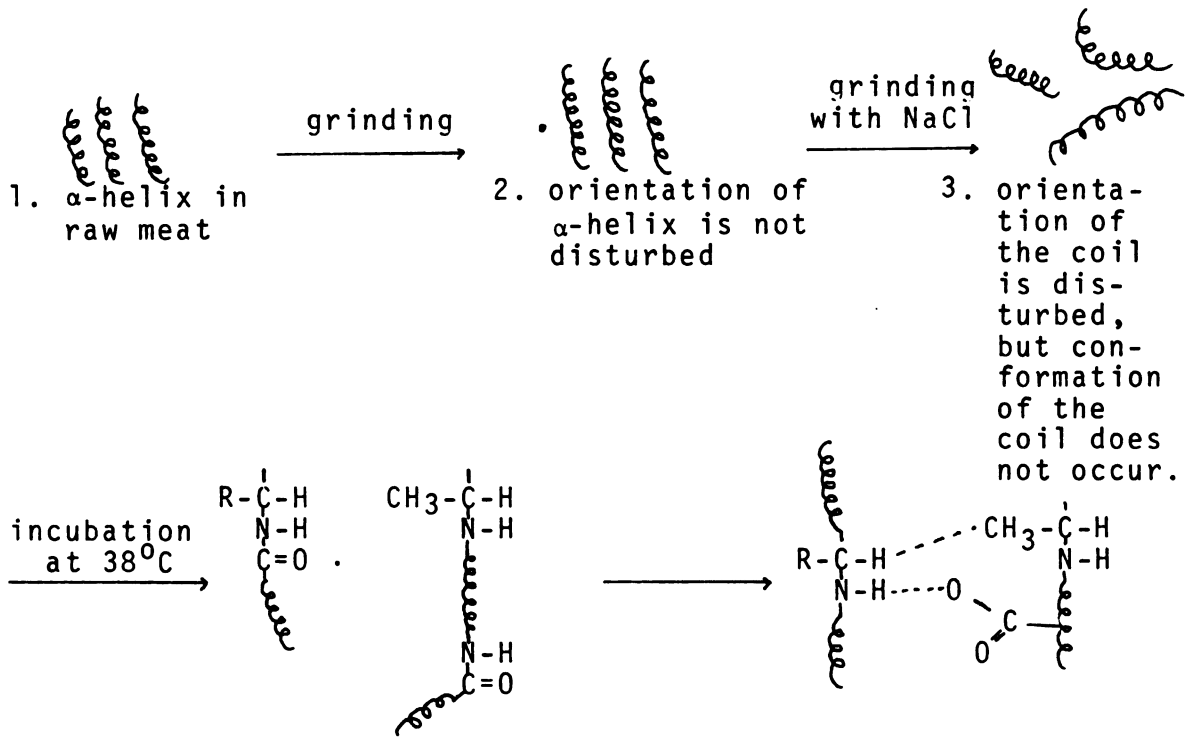
Takagi (1973b), in his further study, indicated that kamaboko is an irreversible hydrogel formed by heat denaturation that contains some proteins other than the myofibrillar proteins. Myofibrillar proteins are distinctive components of the stable cross-linked network in kamaboko. When brayed fish muscle is heated, it changes into a rubber-like kamaboko in which cross-linking and entanglements are formed. However, dense entanglements between network segments which are loosened in the earlier time scale of tensile stress relaxation are obstructed when water soluble protein coexists with the network-forming proteins.

Miyake (1965) employed electron microscopy to observe the muscle destruction during the processing of fish sausage. He concluded that mechanical grinding could not disperse myofilaments from myofibril; and addition of sodium chloride caused rapid dispersion of myofilaments from swollen myofibril. These facts suggested that in fish paste, swelled and well dispersed filaments are held together loosely; while in the heat coagulated product, protein filaments build a kind of network structure holding water, being connected with newly formed free radicals.

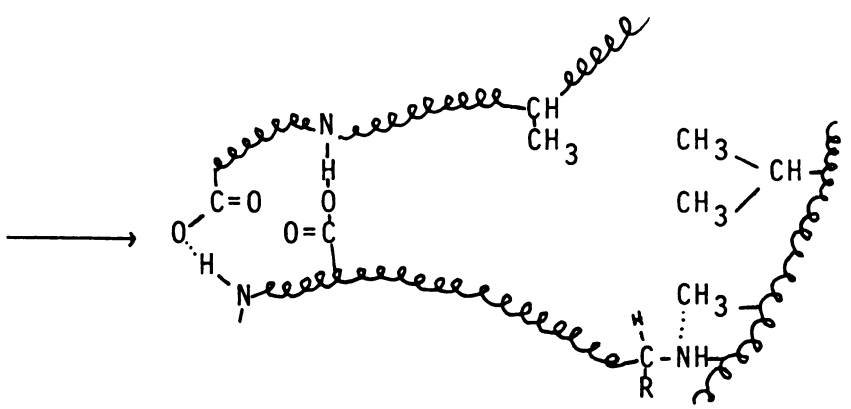
Miyake et al. (1971) studied the fine structure of kamaboko by electron microscopy. They found that the dispersed phase of kamaboko of high elasticity differs from those of low elasticity. Insufficient grinding of fish

paste before heating resulted in a kamaboko of less fine structure; in which the z-lines still remained and between them were found the aggregates of myofilaments. Sufficient grinding of fish muscle resulted in a finer structure of kamaboko; segments of the z-lines, aggregates of myofilaments and fine filaments were well dispersed. These facts suggested that the myofilaments of the network structure might be the aggregates of polymers of myosin, binding with actin filaments which extend on each side of the segments of the z-lines.

The mechanism of setting (suwari) and deterioration (modori) phenomena that take place in fish paste products was postulated by Niwa and Miyake (1971a). They found that the actomyosin was related to the setting phenomenon, in which no changes in the conformation of polypeptides were observed in either raw meat or ground meat; a slight disordered arrangement of protein molecules was detected when meat was ground with sodium chloride, though a α-helical structure was retained. A part of the α-helical structure of polypeptides was transformed into a random coiled one at the advanced stage of setting. A possible scheme for gel formation at the setting process is illustrated as follows (Niwa and Miyake, 1971b):



1. α-helix in raw meat
2. orientation of α-helix is not disturbed
3. orientation of the coil is disturbed, but conformation of the coil does not occur.
4. Trans conformation of α-helix to random coil begins to occur.
5. Aggregation of the polypeptide chains.



6. Formation of protein network, the α-helix of the chain still remains.

Tanikawa (1971) suggested that the elasticity of cooked fish gels are dependent upon several factors including the quantity and quality of myofibrillar proteins, fat content, fishing season and location, pH of the preblended fish muscle, kind of fish muscle (white or dark), physiological state of fish muscle (prerigor, on-set of rigor, or post rigor), freezing of fish and addition of ingredients or additives in the preblended fish muscle (e.g. sodium chloride, phosphate, sugar, starch and oil etc.).

Tanikawa (1971) pointed out that fish of white muscle having high myosin and low fat content produces better gel elasticity than those of red muscle and/or fish from spawning season due to their high fat content. Addition of oil to the minced flesh or the fat from tissue itself tends to dilute the myosin content in the pre-blended fish paste or mixture that causes the weakening of gel strength. Products made from prerigor fish had better gel elasticity than those from rigor and post-rigor muscle. However, the elasticity of croaker and shark, is not weakened even after loss of freshness. Aged shark produced stronger gel elasticity than those from the fresh shark. This is probably due to their longer postmortem period and higher stroma protein content that affected the myosin extractability. The strongest elasticity is obtained from the fish muscle having a pH value between 6.5 to 7.0. The optimum pH of dark muscle fish for the formation of gel elasticity is 6.2 to 6.7, and

for white muscle fish is 7.0 to 7.5. The basic amino acids having pH values of 7.5 to 8.1 up to 0.2 to 0.5% of the total fish paste provides stronger gel elasticity. Freezing of fish caused denaturation of muscle proteins and loss of water holding capacity; as a consequence, it brought about reduced gel forming ability. Addition of 3% salt and 0.3% sodium polyphosphate increased the extractability of myofibrillar proteins, therefore, bringing about enhanced gel elasticity. Addition of starch and/or gluten enforced gel strength. Excessive disruption of myofibrils by the grinding action or mechanical mincing resulted in decreased water holding capacity which reduced gel forming ability.

EXPERIMENTAL

Food Materials and Ingredients

White suckers used in this study were harvested commercially by trap net from Saginaw Bay, Lake Huron. Two batches were purchased from Bay Port Fish Co., Bay Port, Michigan on November 18 and 22, 1980. Suckers were boxed in ice for 0 to 3 days at time of purchase, were re-iced and transported without additional refrigeration to the Meat Laboratory of the Michigan State University. Suckers were stored at 2°C and processed the next day. Most suckers weighed in the range of 1 to 2 pounds. The total suckers from each batch weighed approximately 250 pounds. Suckers used for fractionation of myofibrillar proteins in experiment I were boxed in ice while they were still living, and immediately shipped within 3 hours to the food laboratory of the Michigan State University, afterwhich they were stored at 2°C for 0 to 6 days.

Most of the dry ingredients were obtained from the local stores. Potato starch was purchased from Randall Health Foods, East Lansing, Michigan. Sucrose, salt, monosodium glutamate and soy sauce were bought from Meijer Thrifty Acres, Okemos, Michigan. Instant onion and lamb

sauce were donated by Dr. L.J. Minor from L.J. Minor's Corp., Cleveland, Ohio. Sodium hexametaphosphate was donated by FMC. Co., Philadelphia, Pennsylvania.

Chemicals and Laboratory Materials

Solvents and Chemicals

All chemicals used in this study were reagent grade. All solvents and acids were freshly redistilled before use. They were all purchased from the general store of Michigan State University.

Reference Standards

Standard mixture of fatty acid methyl esters, standard polar lipid mixtures, lyso polar lipid mixture, and β-cholestane standards were obtained from Supelco Inc., Bellefonte, PA.

Both high and low molecular protein standard kits were obtained from Pharmacia Fine Chemicals. Standard protein solution for Biuret analyses was obtained from Sigma Chemical Co., Missouri.

Hypoxanthine anhydrous and xanthine oxidase were obtained from Sigma Chemical Co. where hypoxanthine anhydrous was used to prepare a series of hypoxanthine standard solutions.

Others

Column packing materials for gas liquid chromatography were obtained from Supelco Co. Precoated silica gel G plates for thin layer chromatograph (TLC) were obtained from Applied Science Laboratories Inc. Methanolic-base and BF_3-Methanol were obtained from Supelco Co.

Methods

This study is divided into two parts. The first part of this experiment was conducted to determine the effect of the freshness of sucker flesh as well as the washing technique on the extractability of salt soluble proteins, and their composition as well as the gel forming ability of the minced sucker flesh. The second part of this study involved the effect of washing technique on the lipid composition, cholesterol level, as well as TBA values; and further determination of the effect of additives and frozen storage on lipid stability of both raw fish pastes and cooked fish balls during frozen storage at -23°C for six months. Duplicate determinations were performed for all chemical tests.

Experiment I

The sucker flesh used in this part of the study was prepared from five suckers daily by a hand-deboning operation. The deboned sucker flesh was ground through a Kitchen Aid Meat Grinder, Model K-5A. The freshly ground sucker flesh

was immediately assigned for hypoxanthine test and extraction of myofibrillar proteins. The sucker flesh used for gel forming ability was held in a bag made of 4 layers of cheese cloth and washed in a Kenmore household clothes washer, using a gentle cycle and spun at regular cycle. The purpose of washing and spinning was to remove the sarcoplasmic constituents, blood residues, unbound lipids and the excessive water since these materials are factors that would weaken the gel strength of the cooked fish paste. The washed sucker flesh was blended with salt, monosodium glutamate, sucrose, sodium hexametaphosphate and potato starch; afterwhich the blended fish paste was made into fish balls following the procedure and formulations shown in Figure 1 and Table 2 (Experiment II).

Moisture Analysis

Moisture content determination for unwashed sucker flesh was carried out in a Hotpack vacuum oven, model 633, at 100^{o}C and a vacuum of 27 in Hg following the official AOAC method (1975). Samples were dried to a constant weight

for 10 hrs and the dried weight was converted to the percentage of the wet weight. For the routine test, the moisture content of the washed minced sucker flesh was determined in a microwave oven in order to obtain a value that is close to the unwashed sample (±0.5%) at a rapid speed, so that the processing of fish paste could be continued soon after washing.

Quantitation of Total Salt Extractable Muscle Protein

The determination of total salt extractable muscle proteins from ice-stored suckers was conducted according to the method described by Iwata and Okada (1971) as follows: duplicate portions of 20 g coarsely minced sucker flesh were homogenized with 180 ml of 0.45 M KCl-phosphate buffer solution (I = 0.5, pH 7.2) for 90 sec, respectively. The homogenates were allowed to stand at room temperature for one hour; after which, the homogenates were centrifuged at 15,000 x g for 30 min and filtered through 8 layers of cheese cloth. The total volume of the supernatant was measured and recorded. Total protein concentration in the supernatant was determined by using micro-Kjeldahl analysis.

Fish Muscle Protein Fractionation

The procedure for fish muscle protein fractionation was that reported by Cheng et al. (1979). Duplicate samples were extracted twice. Extractions were carried out at 2^{o} to 3^{o}C with cold extracting solutions.

Three solutions were prepared for muscle protein fractionation: solution A contained 0.1 M NaCl and 0.02 M sodium phosphate buffered at pH 7.0; solution B contained 0.6 M NaCl and 0.1 M sodium phosphate buffered at pH 7.0; solution C contained 20% trichloroacetic acid (TCA).

Sarcoplasmic proteins were prepared by homogenizing 20 g minced sucker with 200 ml solution A for three 15 second periods at 5 second intervals and centrifuging at 15,000 x g for 15 minutes. The homogenates were filtered through 8 layers of cheese cloth. The supernatant contained mainly sarcoplasmic proteins and minor non-protein-nitrogen materials. The residues which remained on the cheese cloth were suspended in 150 ml of solution A for 30 min at 4^{o}C. The suspensions were centrifuged at 15,000 x g for 20 min and filtered through 8 layers of cheese cloth. The second supernatant was collected and combined with the first supernatant. The total volume of supernatant was measured and recorded.

Myofibrillar proteins prepared from the residue of sarcoplasmic fractionation were suspended in 200 ml of solution B and extracted for 3 hours at refrigerated temperature by placing the container in a sodium chloride-ice slurry bath. The solutions containing muscle protein residues were stirred constantly with a magnetic stirrer. They were centrifuged at 15,000 x g for 15 min and filtered through 8 layers of cheese cloth. The supernatants were

collected, and the residues were resuspended in 100 ml solution B for another 30 min with constant stirring in the same manner as the former step. The second supernatants were collected and combined with the first one. The total volume of myofibrillar protein supernatant was measured and recorded.

Non-protein-nitrogen (NPN) was prepared by mixing the sarcoplasmic supernatant with an equal volume of 20% TCA. The mixture was allowed to stand overnight in the refrigerator and filtered through 8 layers of cheese cloth. The supernatant contained NPN. The total volume of NPN was measured and recorded. The nitrogen content of the sarcoplasmic fraction, NPN, and myofibrillar proteins were determined by the micro-Kjeldahl method (AOAC, 1975).

Preparation of Salt (Sodium Chloride) Extractable Protein for Sodium Dodecylsulfate (SDS) Polyacrylamide Gel Electrophoresis

Two portions of 40 g minced suckers were extracted with 200 ml 0.17% NaCl solution and stirred constantly with an electric magnet stirrer at 4^{o}C. The suspensions were filtered through 8 layers of cheese cloth and washed with 400 ml of 0.17% NaCl solution while filtering. The supernatant was discarded. The residues were then resuspended with 200 ml of 3.9% salt solution and homogenized in a Waring blender for 30 seconds. The homogenates were

centrifuged at 14,500 x g for 20 min and filtered through 8 layers of cheese cloth. The supernatants were collected. The residues were resuspended in 200 ml 3.9% salt solution with constant stirring by a magnetic stirrer for 5 min at 4°C. The solution was centrifuged and filtered and the supernatant was collected. Both supernatants were combined. The total volume of the supernatant was measured and recorded.

The protein concentration of the salt extractable protein was determined by the Biuret method described by Layne (1957).

The freshly extracted salt-soluble protein was mixed with an equal volume of glycerol and stored at -18°C for sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE was conducted by the method of Weber and Osborne (1969). The following solutions were prepared for SDS-PAGE: (1) Tris-glycine stock solution (0.5 M Tris and 1.5 M glycine), (2) 25% acrylamide, 0.25% N,N-methylene-bisacrylamide (Bis) solution, (3) 2.5% sodium dodecylsulfate (SDS) solution, (4) 1% ammonium persulfate solution, (5) chember buffer solution (0.1% SDS, 0.2 M Tris-glycine, pH 8.8), (6) tracking dye solution, (7) staining solution and (8) destaining solution. The detailed methods and formulations of these solutions are shown in Appendix A.

Acrylamide (10%) gels were prepared by using 10 ml 25% acrylamide Bis, 5 ml tris-glycine buffer, 1.25 ml glycerol

and 6.75 ml of deionized water was pipetted into a 40 ml glass-stoppered flask which was connected with a vacuum degassing apparatus. The mixture was well mixed by an electric magnetic stirrer during degassing. After degassing, the following solutions were added in this order: 1 ml 2.5% SDS, 0.01 ml TEMED and 1 ml 1% ammonium persulfate. The solutions were mixed gently by swirling briefly to avoid formation of air bubbles and foam. The solution was immediately transferred to the treated glass tubes by using a 10 ml syringe. The tubes were filled up to a height of 8 cm. Tubes with gels were carefully overlayed with water to about a height of 1 cm, and were allowed to polymerize without disturbing for 20 to 30 minutes. After the gels had solidified, the top water was decanted. The tube-stoppers were removed from the bottom of the tubes.

Protein solution for SDS-PAGE was prepared by mixing the salt soluble proteins and glycerol and diluted with tracking dye buffered solution to contain 0.4 mg protein/ml. The tracking dye-protein mixture was heated in a boiling water bath for 5 min. Standard protein mixtures of both high and low molecular weight were prepared in the same manner as the salt soluble proteins. The chamber buffered solution was filled to two-thirds of the lower chamber. The tubes containing polymerized gels were loaded on the electrophoresis chamber. The same buffered solution was filled in the upper chamber to 1 cm above the tubes. All

the air bubbles that formed on the upper end of the tubes were expelled carefully by using a glass-rod without breaking the gels. Each gel was then loaded with 30 μl sample. The entry of the sample into the gels was conducted at a current of 0.2 mA per gel. After the dye had completely entered, the current was increased to 0.5 mA per gel and the migration continued until the dye front reached to 1 cm above from the end of the tube. It took approximately 10 to 12 hours for the total run. The gels were removed from running tubes by squirting water from a syringe between the edge of the gel and the tube wall. They were immersed in staining solution for 10 hours, followed by destaining in a diffusion chamber Model 172A for 3 days.

Gels were scanned using a Beckman DB-G Grating Spectrophotometer Model 2400 equipped with a Gilford Gel Scanner (Model 2520) and a Photometer 252. This system was connected with a Hewlett-Packard Integrator (Model 33805). The gels were scanned at a rate of 1.0 cm/min and a chart speed of 2.0 cm/min start delay and slope sensitivity settings were 0 and 3.0 mV/min, respectively. SDS-PAGE gels were scanned at a wavelength of 550 nm. The relative areas of individual protein peaks were recorded. The relative mobility of the bands was assessed from the total length of the gel, tracking dye migration distance and from the distances migrated by individual proteins. A standard molecular weight calibration curve was established by plotting the log

molecular weights of standard proteins versus their relative mobilities on semi-log paper. The unknown proteins were identified by comparing their relative mobilities to that of the standard proteins.

Experiment II

Sucker flesh used for experiment II was prepared by using a mechanical deboner as described in the following section. Treatments for this experiment were designed to assess the lipid stability among the washed samples pre-blended with salt, sucrose, monosodium glutamate (MSG), sodium hexametaphosphate (SHMP) and potato starch as shown in Table 3. The shelf life of the raw fish paste and/or cooked fish balls were monitored by analyses of lipid oxidation using TBA test and chromatographic methods, in a system, where lipids and proteins play a concurrent action during frozen storage. In addition, preliminary experiments were conducted to quantitate the effect of washing on the lipid compounds and stability.

Preparation of Minced Suckers and Mechanical Deboner Operation

Suckers were manually deheaded, gutted and split dorso-ventrally parallel to the backbone. The split suckers were washed under cool running tap water, using a hand brush to facilitate removal of liver, intestine and kidney material. They were immediately layered with crushed ice until being deboned within half an hour.

The mechanical deboner was a Bibun Model SD13 (Bibun Co., Fukuyama Kiroshima, Japan) belt type machine equipped with a 3 mm perforation size drum. The dressed suckers were passed through the machine flesh side to the drum and the minced flesh was recovered in a plastic lug. The minced sucker was passed twice through the deboner in order to insure that a minimum of small bone residue and scales remained in the minced flesh. The minced flesh yield after mechanical deboning was approximately 49% of the round weight.

Washing of the Minced Sucker Flesh

Samples of minced flesh were collected after the second pass through the mechanical deboner (unwashed samples) and analyzed for moisture, total fat, protein, nonprotein nitrogen and TBA. The remaining minced sucker flesh was weighed into 2.5 lbs aliquots and placed in bags made of 4 layers of cheese cloth. Four bags of minced sucker were washed per load in a Kenmore household clothes washer using a gentle cycle and spun at regular cycle. Washing and spinning not only removed the excessive amount of water but also removed the undesirable sarcoplasmic constituents, contaminants such as blood and internal organs, and the unbound lipids which cause degradation of both protein and lipid components in the sucker flesh. Consequently, it brings about the weakening of the gel strength and undesirable flavor of the cooked

products. The washed samples were stored at 2°C in the refrigerator for further processing into fish paste and fish balls. These products were vacuum packed in one pound lots in cryovac (polyvinylidene chloride) bags which were then stored at -23°C for 0 to 6 months. The average yields of washed samples were approximately 55% of the minced sample. Analyses of the minced unwashed sucker flesh showed a moisture content of 80.75% and a protein content of 16.00%.

Formulation of Fish Paste and/or Fish Balls

In this study, the formulation of fish pastes (Table 3) was derived from the formulation of Kamaboko as shown in Table 2. Kamaboko is a steamed Japanese style fish paste product.

Hwang and Jeng (1979) reported that fish balls containing less than 6% starch and less than 4% sugar and fat were highly accepted by sensory evaluation. Products with 3% salt were indicated too salty by pretesting panels. Therefore, in this study, the level of salt was adjusted to 2.5%. This level of salt allowed extraction of 95% total myosin in the fish paste system. According to Noguchi and Matumoto (1970), 0.3 M Na-glutamate is as effective as 1 M glucose in preventing the denaturation of myofibrillar proteins as well as in producing high gel-strength of kamaboko. The preventive effect of Na-glutamate on protein denaturation was found to approach the maximum beyond 0.025 M (0.42%). By computing the 0.025 M ratio of Na-glutamate to sucrose, a

Table 2. Formulation of kamaboko.[a]

Ingredients	%/100 g fish
Minced fish	100.00
Salt	3.01-3.15
Sodium glutamate	0.21-0.40
Sugar	4.53-14.72
Starch	3.15-14.72
Sweet sake	2.40
Egg white (or	8.53
Polyphosphate)	0.2-0.3%
Water	0% if starch is 10%
	5% if starch is 15 to 20%

[a]Tanikawa (1971).

Table 3. Formulation of fish paste and/or fish balls.

Ingredients	%/100 g minced fish
Washed, minced sucker	100.00
Salt (sodium chloride)	2.50
Monosodium glutamate (MSG)	0.40
Sucrose	2.00
Sodium hexametaphosphate	0.30
Potato starch	10.00

0.08 M (2.74% sucrose has an equivalent effect as 0.025 M Na-glutamate. In this study, the formulation of Chinese style fish paste was adjusted for these requirements as previous researchers had suggested. Formulation of raw fish paste and/or fish ball is shown in Table 3. According to the Food Chemical Codex, 0.3% sodium hexametaphosphate is under the maximum allowance (0.5%) as a food additive by USDA regulations (Code of Federal Regulation, 1971). At this level, it gives the maximum swelling ability of myofibrillar proteins in a gel type product.

Processing of Fish Pastes and Fish Balls

There are 3 consecutive steps of processing fish pastes as shown in the following flow chart (Figure 1).

Duplicate samples of four portions of 2,400 g washed minced sucker flesh were blended with four series of mixed additives (Table 4) separately for preparing fish pastes. These 4 additive mixtures are: (1) 2.5% sodium chloride, 0.4% MSG, 0.3% sodium hexametaphosphate, 10% potato starch, (2) 2.5% sodium chloride, 0.4% MSG, 2% sucrose, 10% potato starch, (3) 0.4% MSG, 2% sucrose, 0.3% sodium hexametaphosphate, 10% potato starch, (4) 2.5% sodium chloride, 0.4% MSG, 2% sucrose, 0.3% sodium hexametaphosphate and 10% potato starch.

Duplicates of 7.000 g washed minced sucker flesh were also blended with mixed additives of 2.5% sodium chloride,

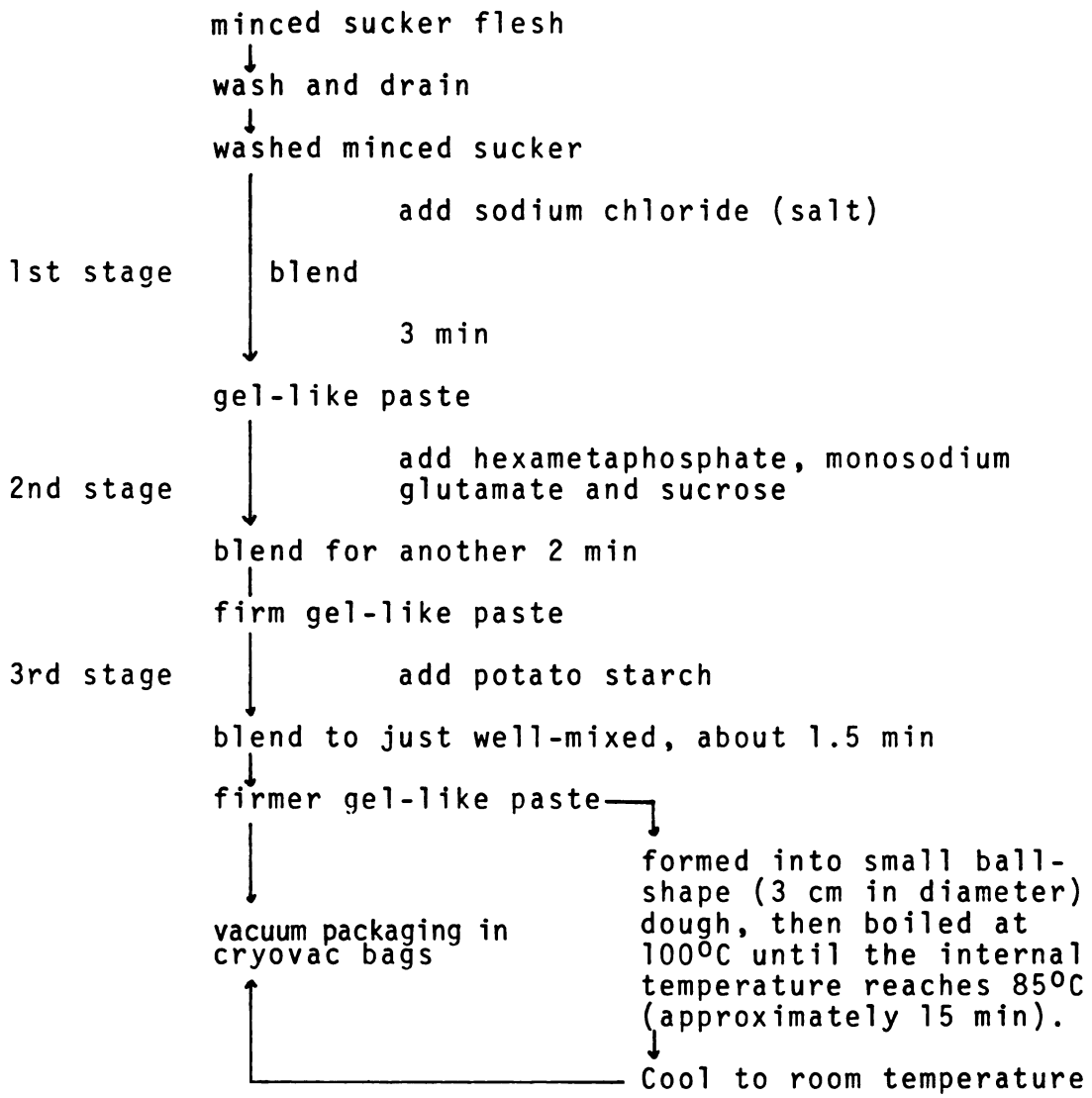


Figure 1. Flow chart for processing fish paste and fish balls.

Table 4. Design of treatment on washed minced sucker pastes.

Code for Treatment	Percent Ingredient Per 100 g Minced Sucker					Combination of Treatment
	Sodium Chloride (NaCl)	Monosodium Glutamate (MSG)	Sucrose	Sodium Hexameta-phosphate (SHMP)	Potato Starch	
Raw Fish Pastes						
#1	2.5	0.4	2.0	-	10.0	Without SHMP
#2	2.5	0.4	-	0.3	10.0	Without Sucrose
#3	-	0.4	2.0	0.3	10.0	Without NaCl
#4	2.5	0.4	2.0	0.3	10.0	With all ingredients
Cooked Fish Balls	2.5	0.4	2.0	0.3	10.0	With all ingredients and heat treatment

0.4% MSG, 2% sucrose, 0.3% sodium hexametaphosphate and 10% potato starch for making fish balls.

Minced fish, additives and starch were blended in a Kitchen Aid mixer (Model K5-A), at 168 rpm speed for fish pastes. Ingredients for fish balls were blended in a Kitchen Aid Mixer (Model N-50, Hobart Mfg. Co., Troy, Ohio).

At first stage, the minced flesh and sodium chloride were blended for 3 min or until a gel was developed which ensured all the myofibrillar proteins, especially myosin was extracted by salt. At the second stage, hexametaphosphate, sucrose and monosodium glutamate were added, blending continued for another 2 minutes to allow the swelling of muscle proteins and even distribution of the myofibrillar proteins throughout the entire network of the gel-like paste. At the third stage, potato starch was added to enhance the gel strength of the fish paste.

Extraction of Total Lipids

The procedure of extraction of lipids from minced sucker flesh was essentially based on the method of Bligh and Dyer (1959), modified by Peng and Dugan (1965) who adapted the washing system from Folch et al. (1957). The samples of frozen sucker pastes and fish balls were defrosted in a microwave oven for 4 min or until the internal temperature reached 2^{o}C right before lipid extraction. Each 100 g of freshly minced sucker (both washed and unwashed), unfrozen

fish pastes and fish balls, and defrosted samples were homogenized in a Waring blender for 2 minutes with a solvent system containing 100 ml of chloroform, 200 ml of methanol, and deionized water to make the total moisture content equivalent to 80% of the sample weight. Then 100 ml of chloroform was added to the mixture. Blending continued for 30 seconds, 100 ml of deionized water was added and blending continued for another 30 seconds. The homogenate was filtered through Whatman No. 1 paper on a porcelain Buchner funnel which was fitted on a filter flask with slight vacuum suction to ensure maximum recovery of solvent. The homogenate residue and filter paper were blended with 200 ml of chloroform for 1 minute and filtered through the same Buchner funnel. The blender was then rinsed with 50 ml of chloroform and 40 ml of methanol, separately. The collected filtrates were transferred quantitatively to a 1000 ml separatory funnel. An 0.2% potassium chloride solution (0.74% w/v) of the total filtrate (v/v) was added to the filtrate and mixed well to facilitate the separation of the water soluble protein component from the fat soluble phase. The mixture was left in a freezer at -23^{o}C overnight for complete extraction and separation. The lower phase, comprised mainly of chloroform soluble lipids, consisted approximately of chloroform: methanol:water = 86:14:1. The lower phase was collected and filtered through a glass funnel lined with No. 4 Whatman filter paper containing approximately 30 g of anhydrous

sodium sulfate into a glass stoppered round bottom flask. The upper phase (mainly water soluble components and minor methanol soluble lipid components) was washed with 3 portions of 30 ml chloroform, the lower chloroform phase was collected and filtered in the same manner as in the first chloroform soluble phase. These two extractions were combined. The total volume of the chloroform phase was measured and recorded. Duplicate 10 ml aliquots were taken from the chloroform-lipid extracts into weighed Erlenmeyer flasks, the solvent was evaporated by allowing the Erlenmeyer flasks to stand on the top of a steam bath until the volume was reduced to approximately 1 ml, and the flasks were placed in a vacuum oven at 70^{o}C for 2 hours to complete the solvent evaporation. The flasks with fat residue were then put into a desiccator over calcium chloride for 24 hours and weighed. The percentage of lipid content in the extracts was computed from their weight differences.

Concentration of the Lipid Extracts

The chloroform phase was held in a 500 ml round bottom flask and evaporated by a Rinco rotating high vacuum type evaporator almost to dryness, and transferred to a weighed glass vial fitted with a teflon lined screw cap. The flask was rinsed with small amounts of chloroform with the aid of a disposable pipet, and combined with the concentrated lipid in the same vial. The lipid containing small amounts of

Preparation of silicic acid columns. For each column (2.5x25 cm.) a 60 ml of silicic acid slurry was prepared by mixing 30 g of activated silicic acid and 60 ml of chloroform in a filtering flask with the aid of a magnetic stirrer until a homogeneous translucent mixture was obtained. The silicic acid slurry was then degassed by a vacuum set-up which consisted of an additional trapping flask, to avoid water entering into the flask containing the silicic acid slurry. The column containing 10 ml of chloroform was packed with degassed slurry, by pouring the slurry slowly into the column along the edge of a glass rod, in such a way that no air bubbles would be trapped. The silicic acid was allowed to settle and elute first with chloroform, followed with methanol and finally with chloroform, to check whether undesirable air bubbles and channeling existed in the column. A thin layer of glass-wool and 2 cm layer of anhydrous sodium sulfate were laid on top of the silicic layer. The column was ready for lipid fractionation. Since the packed silicic column should never be allowed to dry out, the solvent layer was kept 3 cm above the sodium sulfate layer, so that the undesirable channeling could be avoided.

Approximately 0.5 g of lipid extracts were redissolved in 1-2 ml of chloroform and applied onto the column. The loading capacity of silicic acid for lipid is approximately 0.02 g lipid per gram silicic acid. The column flow rate was adjusted to approximately 3 ml per minute by applying a

constant pressure with a stream of nitrogen gas on top of the column. Neutral lipids were eluted first with chloroform until a negative Salkowski test was achieved, in which 1 ml of chloroform-lipid eluent was carefully added to an equal volume of concentrated sulfuric acid in a test tube. The development of a characteristic yellow to brown band in the test tube indicated the presence of lipid in the chloroform. Acetone, which acted as a scavenger for oxidized materials (Peng, 1965), was eluted next to remove pigmented materials. Phospholipids were eluted with methanol until a negative ninhydrin test was reached. Purity of neutral lipid was further tested by the ninhydrin test, and the phospholipids by micro thin layer chromatography with a developing system containing petroleum ether:ethyl ether:acetic acid (90:10:1 by volume). The slides were sprayed with sulfuric acid followed by charring. Migration of the original spot, indicated presence of neutral lipids.

The ninhydrin test was done by adding ninhydrin to the methanol eluant in a 15 ml vial, covered with a teflon screw cap, placed on a block of heating module at 150^{o}C, and shaken vigorously periodically. The development of a purplish color indicated the presence of phosphatidyl ethanolamine and its lyso derivatives.

The neutral and phospholipid fractions were used to prepare fatty acids for esterification, or dried for gravimetric determination of total neutral and phospholipids.

Total lipid was calculated from the sum of these two values.

Classification of Phospholipids

Approximately 100 mg of phospholipids were dissolved in chloroform and separated into their components using preparative thin layer chromatography (TLC). Twenty microliter of phospholipid solution were spotted on each plate using a stream of nitrogen gas using a Hamilton microsyringe. A standard polar lipid mixture (cholesterol, phosphatidyl ethanolamine, phosphatidyl choline) and standard lysopolar lipid mixture (lysophosphatidyl ethanolamine, lysophosphatidyl serine, lysophosphatidyl choline) were spotted on the right hand side of the plate. The plates were developed in a standard TLC developing tank saturated with solvents containing chloroform:methanol:water:acetic acid - 60:30:5:0.5. After the solvent had ascended 17 cm on the plates, the plates were removed from the TLC tank and allowed to dry at room temperature under the ventilation hood. The spots of lipid components were detected by spraying with different indicators: e.g., ninhydrin solution for phosphatidyl ethanolamine and Dragendorf agent for choline (Skidmore and Entenman, 1962). The relative Rf values in this developing system were found to be 0.80 for phosphatidyl ethanolamine, 0.47 for phosphatidyl choline (lecithin), 0.15 for lysophosphatidyl ethanolamine, and 0.07 for lysophosphatidyl choline.

Preparation of Methyl Esters

Methylation of total lipids, neutral lipids and phospholipids from all minced sucker products were conducted according to the method described by Morrison and Smith (1964). Lipids were converted to methyl esters by treatment of lipids with boron trifluoride-methanol reagent (14% BF_3 in methanol), in which BF_3-methanol acts as acid catalyst in the esterification of fatty acids. The methylated fatty acid esters was preserved under nitrogen and covered tightly and stored at -23^{o}C before injecting into a gas chromatograph.

Total Cholesterol Determination

Total cholesterol level present in total lipid extracts was determined by using the base-catalyzed transesterification method according to Luddy et al. (1960, 1968). Anhydrous methanolic-base (0.5 N sodium methoxide) obtained from Supelco Inc. was used for transesterification of the lipid sample, in which methanolic-base acted as catalyst converting cholesterol into cholesteryl esters, glycerides and phospholipids quantitatively to their methyl esters. The procedure is as follows: into a 15 ml teflon lined screw cap vial were placed 30 mg of total lipids, 1 ml of benzene and 1 ml of the methanolic-base reagent. The vials was sealed and heated in a temperature block heater at 80^{o}C for 20 min and removed from the heating block. The

vials were allowed to cool to room temperature, and 3 ml of deionized water and 3 ml of diethylether then were added and the resulting solution was mixed. The mixture was allowed to stand until two layers of separation were clear. The top benzene-ether layer was pipetted into another clean vial and was once more washed with 3 ml of deionized water; afterwhich the top phase was dried over anhydrous Na_2SO_4. This extract was ready for analysis by GLC.

Gas Chromatography Analyses of Methyl Esters

The qualitative determination of the components of fatty acid methyl esters and cholesterol ester were conducted using a Hewlett-Packard Model 5830A gas chromatograph equipped with a Hewlett-Packard k8850 Terminal. Nitrogen was used as the carrier gas at a flow rate of 30 ml per minute for analyzing fatty acid and 40 ml per minute for cholesterol. Temperature was programmed from 150^{o} to 210^{o}C at a rate of 2^{o}C per minute for fatty acid analysis, cholesterol was analyzed at 260^{o}C isothermally. Temperatures for flame ionization detector and injection ports for fatty acid determination were maintained at 350^{o}C and 210^{o}C respectively; while the temperatures for cholesterol were 350^{o} and 260^{o}C, respectively. The components of the fatty acid methyl esters were reported as % area of the total lipid esters. A sample of 0.5 µl was injected for each analysis.

Fatty acid methyl esters were identified by comparing their relative retention time with the mixture of standard

fatty acid methyl esters of marine source; or by comparing their relative retention times (relative to methyl palmitate), using a plot of logarithm of the relative retention time versus the number of carbon atoms. The percentage of each fatty acid ester was computed by dividing the individual peak area by the sum of total peak areas.

2-Thiobarbituric Acid (TBA) Test

A distillation method for quantitative determination of malonaldehyde in rancid foods from Tarladgis et al. (1960) was used to determine the oxidative rancidity of minced sucker products. Interfering yellow and orange colors were found in the malonaldehyde-TBA distillates at the second month of frozen storage, indicating that interactions of carbonyl compounds with either carbohydrates or proteins had occurred. Therefore, separation of yellow and orange pigments from the pink malonaldehyde-TBA reactant was conducted according to the method described by Yu and Sinnhuber (1962), prior to measuring their optical density by a Beckman DB-G grating spectrophotometer.

Evaluation of Gel Strength Related Internal Structure

Gel strength was determined by using the Allo-Kramer Shear Press, Model SP12, equipped with a Food Technology Inc. Texturecorder, Model TR3. Three shear press measurements of cooked fish balls were determined on cooked fish

balls made from suckers stored in ice from 0 to 6 days.

Unfrozen fish balls from experiment I were prepared in the afternoon, boiled, cooled, packed in polyvinyl bags and stored at $4^{o}C$ overnight (10 hours). Two fish balls weighing from 46 to 65 g were placed side by side into the cell perpendicular to the slots. A 3000 lb maximum load transducer was used with a range setting of 10 and a downward stroke of 30 seconds. Pounds of force per gram of sample required to shear were calculated as follows:

$$\frac{\text{Pounds of force}}{\text{g sample}} = \frac{\frac{3000\ \text{lb}}{10} \times \frac{\text{peak height}}{100}}{\text{sample weight (g)}}$$

The frozen fish balls from experiment 2 were measured the same way for 6 consecutive months of frozen storage. On each month, samples were defrosted in a microwave oven setting for 3 min before measuring the shear press values.

Sensory Evaluation

Fifteen taste panelists (7 ethnics and 8 Americans) from faculty, staff and students at the Department of Food Science and Human Nutrition evaluated fish balls at the 0, 3rd, and 6th month of storage. Fish balls were sliced, stir-fried and seasoned with soy sauce, lamb sauce and instant onion right before serving. Each panelist was asked to rate the samples from 2 batches on the following parameters: off odor and flavor, textural resilience, and

acceptability on a 9-point scale (Appendix C).

Statistical Analysis

Analyses of variance were computed by submitting raw coded data into STAT 4 packaged program interfaced with the CDC cyber 7500 system at Michigan State University. Bonferroni t statistics and Tukey's test statistics were used to compare the test statistics of mean values among different treatments. Possible correlations among proximate composition of fish muscle proteins and shear force data were determined by measuring the product-moment correlation described by Gill (1978).

RESULTS AND DISCUSSION

The Effects of Freshness of Minced Sucker Flesh and Washing Technique on the Extractability of Myofibrillar Proteins and the Gel Forming Ability of Fish Pastes.

Identification of salt extractable myofibrillar proteins in sucker flesh.

Figure 2 shows the relative mobility of 5 high and 4 low molecular weight proteins used as standard, obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The calibration curve was constructed by plotting relative electrophoretic mobility (R_f) vs. log subunit molecular weights of the myofibrillar proteins from sucker flesh. The molecular weights of the unknown proteins were determined by comparison of the electrophoretic mobility of each unknown protein with the mobility of standard proteins of known molecular weight (Appendix A2). According to this procedure eight major myofibrillar proteins were identified in sucker flesh as shown in Table 5. Figure 3 illustrates the scanning of a SDS-PAGE gel with the major protein bands showing different peaks. The relative concentrations of the myofibrillar

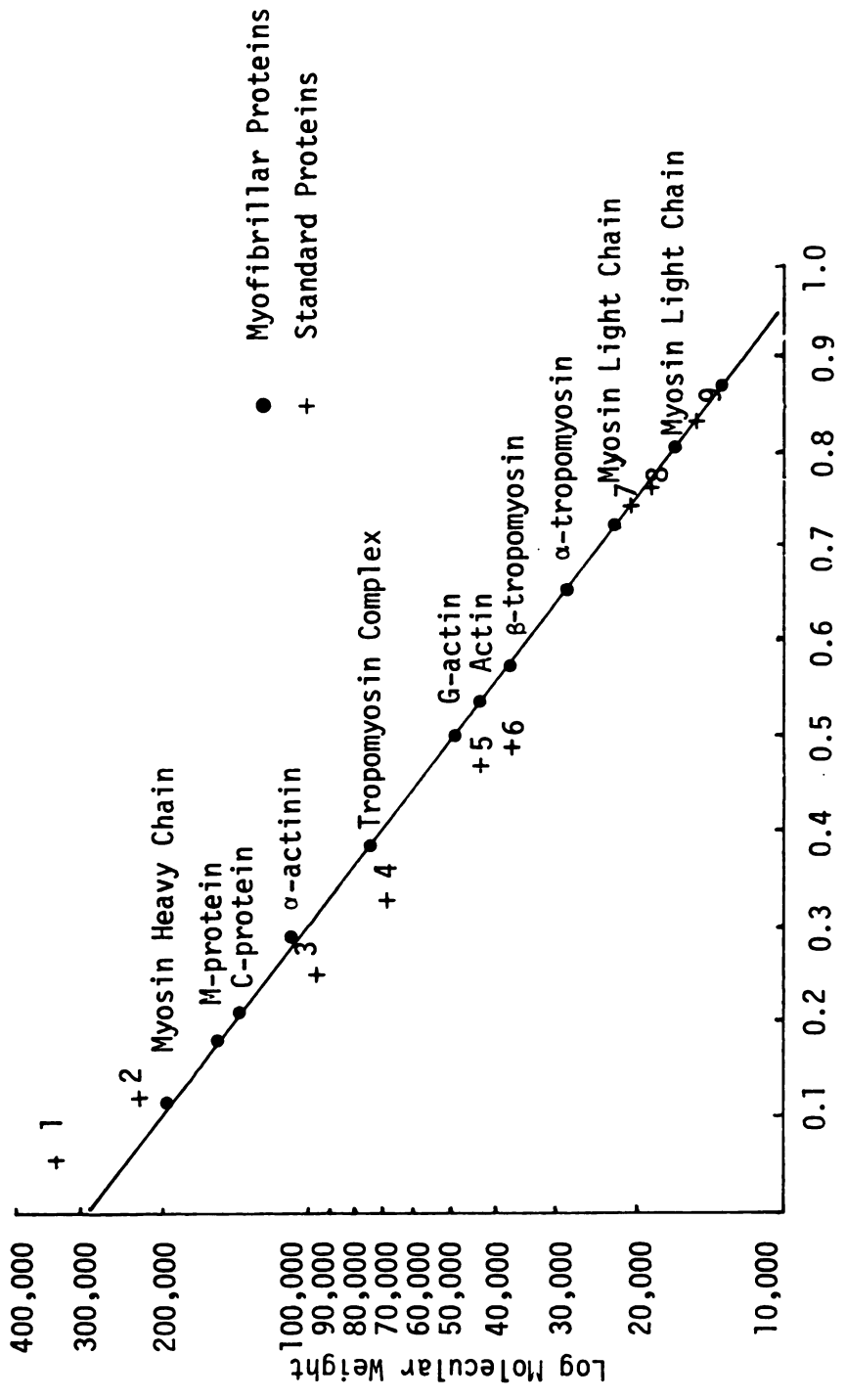


Figure 2. Calibration curve established using high and low molecular calibration kit for SDS-PAGE on a 10% polyacrylamide gel. pH 8.8.

1 = Thyroglobulin
2 = Ferritin
3.= Phosphorylase b
4 = Albumin
5 = Catalase
6 = Lactase dehydrogenase
7 =Carbonic anhydrase
8 =Tyrosin inhibitor
9 = -lactabumin

Table 5. Relative electrophoretic mobility and approximate subunit molecular weight of the major myofibrillar protein in the washed and the unwashed minced sucker flesh that identified on SDS-PAGE[1].

Components of salt extractable proteins	Electrophoretic mobility (R_f)	Molecular weight of subunits (daltons)	Unwashed sample[2] %	Washed sample[2] %
Myosin heavy chain	0.11	200,000	18.32	21.76
Unidentified protein	0.14	180,000	1.87	2.30
Unidentified protein	0.15	170,000	1.45	1.37
Unidentified protein	0.16	165,000	1.96	1.98
M-line protein	0.18	155,000	1.55	1.92
C-protein	0.21	140,000	0.13	0.82
Unidentified protein	0.23	125,000	2.08	5.88
Unidentified protein	0.25	115,000	1.36	-
α-Actinin	0.27	100,000	0.70	0.97
Unidentified	0.31	94,000	2.39	0.51
Tropomysin complex	0.38	70,000	0.88	-
Unidentified	0.41	68,000	1.70	0.43
Unidentified[4]	0.44	57,000	16.80	23.31
G-Actin	0.49	47,000	11.82	2.15
G-Actin	0.53	42,000	9.92	10.91
β-tropomyosin	0.57	35,000	4.62	6.01
α-tropomyosin	0.65	32,000	3.55	5.07
Myosin light chain	0.72	20,000	5.15	6.59
Myosin light chain	0.82	18,500	6.74	7.88
Unidentified protein	0.87	14,000	7.01	0.13

[1]10% polyacrylamide gel in 0.1% SDS.

[2]The unwashed sample was extracted with 3.9% NaCl solution with ionic strength equal to 0.66.

[3]The washed sample was first extracted with 0.17% NaCl solution to remove mainly water soluble components and followed by extraction with 3.9% NaCl solution.

[4]Possible contamination of the sarcoplasmic reticulum proteins and vimentin (a Z-disc protein).

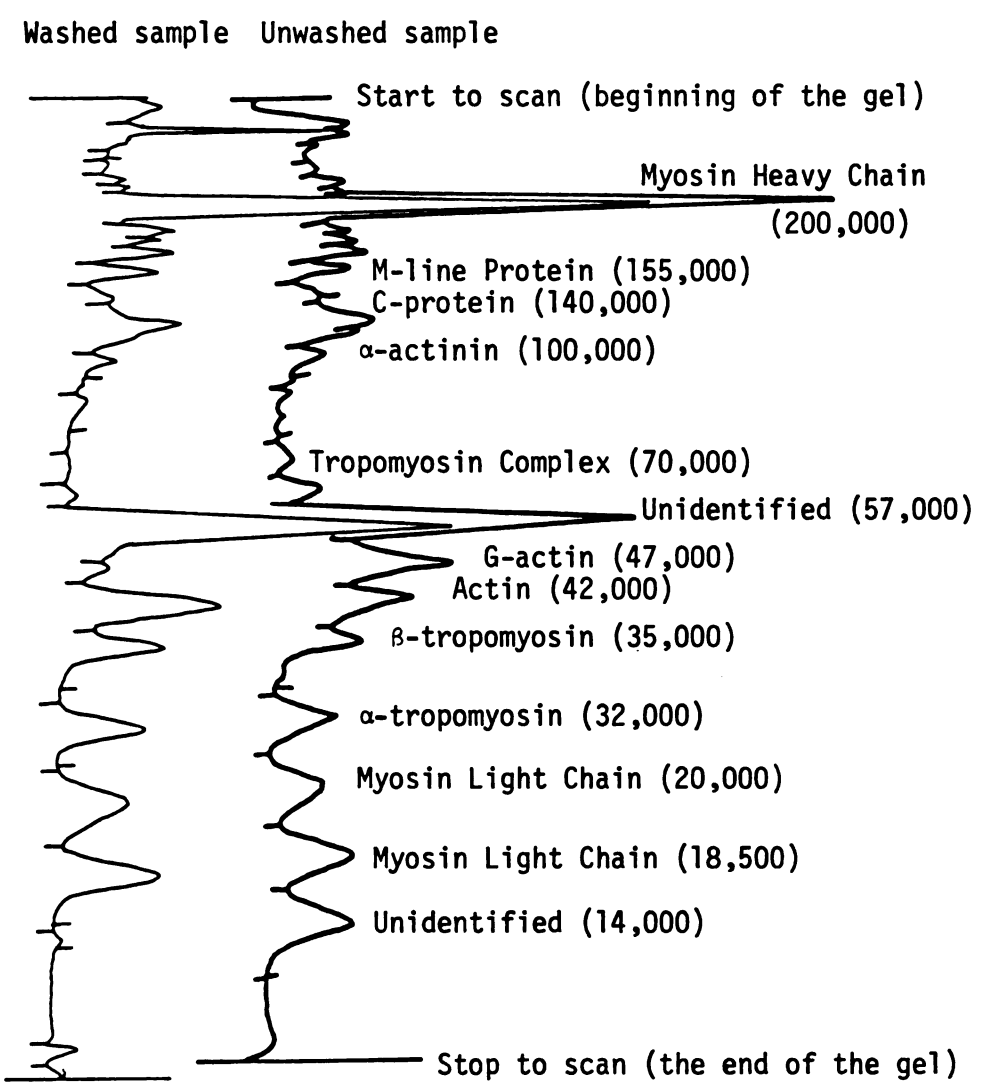


Figure 3. Scanning of SDS-PAGE gels with identification of the major protein bands from washed and unwashed minced sucker muscle (The approximate molecular weights for each identified protein band is given in parentheses).

Table 6. Average percentage and standard deviation of myofibrillar proteins extracted from fresh sucker stored in ice for 0 to 6 days.

Days at ice	Total salt extractable protein nitrogen[1], %	Sarcoplasmic protein nitrogen[2], %	Myofibrillar protein nitrogen[3], %	Non-protein nitrogen[2], %
0	$44.95^{a} \pm 0.35$	$23.76^{a} \pm 0.53$	$16.34^{a} \pm 0.38$	$10.44^{a} \pm 0.11$
1	$31.07^{b} \pm 0.36$	$24.10^{a} \pm 0.36$	$12.63^{c} \pm 0.21$	$10.35^{a} \pm 0.19$
2	$23.68^{e} \pm 0.11$	$24.03^{a} \pm 0.40$	$11.23^{b} \pm 0.12$	$10.67^{a} \pm 0.17$
3	$15.24^{g} \pm 0.23$	$24.16^{a} \pm 0.25$	$10.48^{d} \pm 0.23$	$10.53^{a} \pm 0.25$
4	$18.95^{f} \pm 0.15$	$23.96^{a} \pm 0.26$	$8.49^{e} \pm 0.15$	$10.63^{a} \pm 0.15$
5	$26.04^{d} \pm 0.31$	$23.77^{a} \pm 0.31$	$8.12^{e} \pm 0.06$	$10.43^{a} \pm 0.02$
6	$29.20^{c} \pm 0.29$	$24.11^{a} \pm 0.09$	$5.79^{f} \pm 0.25$	$10.48^{a} \pm 0.23$

[1]Extracted with 0.45M KCl-phosphate buffer solution I=0.5, pH 7.0.

[2]Extracted with 0.1M NaCl and 0.02M sodium phosphate buffered at pH 7.0, I=0.03.

[3]Extracted with 0.6M NaCl and 0.1M phosphate buffer at pH 7.0, I=0.66.

[4]Extracted with 20% trichloroacetic acid solution from sarcoplasmic fraction.

[abcdefg]Figures with different superscripts on the same column are significantly diffweent at $\alpha<0.05$.

proteins were calculated from the area under the band peaks. The effect of washing technique and ice-storage on the extractability of myofibrillar proteins are shown in Tables 5 and 6, respectively. The myosin molecule has been reported to be comprised of one myosin heavy chain and three myosin light chains with subunit molecular weights approximately 21,000, 18,500, and 17,000 daltons. In the present study, only DTNB-light chain (21,000 daltons) and light chain alkali A (18,500 daltons) were retained.

Effect of washing and salt concentration on myofibrillar protein extractability.

Washing minced sucker flesh with 0.1M sodium chloride solution (ionic strength = 0.03) followed by 0.6M salt extraction (ionic strength = 0.66), removed a substantial amount of water soluble constituents as compared with those samples extracted with 0.6M salt solution only. these water soluble constituents included sarcoplasmic proteins, blood materials and proteins with electrophoretic mobilities of 0.25, 0.31, 0.38 (tropomyosin complex), 0.41,0.49, (G-actin) and 0.87 on a 10% polyacrylamide gel (Table 5). This result is in agreement with the finding of Wilkinson et al. (1972). Washing of minced sucker flesh reduced the relative percentage of the extractable G-actin from 11.82% to 2.15%. Loss of G-actin can be explained by the extraction method used which called for

a salt solution on low ionic strength. Seraydarian et al. (1967) reported the depolymerization of F-actin into G-actin and isolation of G-actin was accomplished by extraction at low ionic strength (< 0.001M) at $0^{o}C$ and slightly alkaline pH. In this study, prewashing with 0.17% NaCl solution increased the relative concentration of myosin heavy chain, myosin light chains, M-line protein, C-protein, α-actinin, α-actin, β-tropomyosin, α-tropomyosin and a few unidentified proteins with electrophoretic mobilities of 0.14, 0.23 and 0.44. From these results, it is postulated that prewashing of minced sucker flesh with chilled water would remove more G-actin and other water soluble muscle components than washing with 0.17% salt solution.

In the present study, a 0.6M salt solution with an ionic strength of 0.66 was used to extract the myofibrillar proteins. The freshly minced sucker flesh had pH values ranging from 6.7 to 6.8. The addition of 0.6M sodium chloride to the extraction system caused a drop in pH to 6.2. By increasing the salt concentration, an increase in ionic strength and a decrease in pH of myofibrillar protein was caused. Thus, addition of sodium chloride to the extraction solution would favor the polymerization of F-actin and prevent removal of G-actin from the sucker flesh, causing an increase in the myofibrillar protein extractability.

The lower extractability of myofibrillar proteins at low ionic strength has been attributed to the strong association between myofibrillar proteins (Goll et al., 1970). Dawood (1979) reported that only 13.69% of the total protein of sucker muscle was extracted with plain water. However, a rapid increase in myofibrillar protein extractability occurred on increasing the concentration of sodium chloride from 0 to 3%. This is in agreement with Dyer et al. (1950) who noted that high ionic strength is required to solubilize myofibrillar proteins. Most of the myofibrillar are soluble only in solution with ionic strength ranging from 0.4 to 1.5. In addition to the ionic strength, the pH of the extraction solution and the muscle protein solubility are other factors affecting the muscle protein extractability. Protein solubility increase on both the acidic and basic side of its isoelectric zone which ranges from 5.5 to 6.0 for fish protein (Meinke et al., 1972).

The effect of ice-storage on salt-extractability of myofibrillar proteins.

Data consisting of the total salt extractable proteins, myofibrillar proteins and non-protein nitrogen of sucker flesh stored in ice are presented in Table 6. Orthogonal tests showing significant differences among extractable of salt soluble proteins and myofibrillar proteins during 6 days of ice-storage are presented in Tables 7 and 8,

respectively. The initial total salt extractable muscle protein was 44.95%. The extractability of muscle protein fell to 15.24% by the third day and increased to 29.20% upon further storage in ice.

It is known that total salt extractable proteins are comprised of partial sarcoplasmic proteins and total myofibrillar proteins (Cheng et al., 1979). The myofibrillar protein extractability decreased linearly during a period of 0 to 6 days in ice-storage (Table 6). There were no significant changes in the sarcoplasmic fraction and non-protein nitrogen content during this time (Table 6). This result suggests that a decrease in myofibrillar protein extractability caused a decrease in the ratio of myofibrillar proteins to sarcoplasmic proteins; consequently, this changes would decrease the gel forming ability as shown by Nishimoto and Koreeda (1979). The maximum extractability of myofibrillar proteins was on day 0. Possibly the suckers were still in pre-rigor or at the on-set of rigor since the first extraction was taken within 5 hours of ice-packaging. The lowest levels of total salt extractable proteins were obtained on the third day of storage in ice. This suggests that suckers were at the maximum stage of rigor on this day. As such, muscle fibers demonstrated increased resistance to fragmentation and reduced protein solubility.

Anderson and Ravesi (1970) reported that protein extractability of cod muscle aged in ice decreased at a slower rate than that during frozen storage. Their finding was in

Table 7. Orthogonal test of statistics for extractability of myofibrillar protein in ice-stored sucker flesh.

Source of variation	Degree of freedom	Sum of square	Mean square	F-ratio	$F\alpha, V_1, 21$
Treatment	6	279.87	46.65	2.76[1]	2.08
Linear	1	262.15	262.15	15.49[2]	14.60
Quadratic	1	57.36	57.36	3.39[3]	2.96
Error	21	355.25	16.92		

[1]Significantly different at $\alpha<0.02$.

[2]Significantly different at $\alpha<0.01$.

[3]Significantly different at $\alpha<0.02$.

Table 8. Orthogonal test of statistics for salt-extractable proteins in ice-stored sucker flesh.

Source of variation	Degree of freedom	Sum of square	Mean square	F-ratio	$F0.001, Vi, 21$
Treatment	6	2233.59	372.27	4136.33	5.88
Linear	1	468.84	468.84	5209.33	14.60
Quadratic	1	1575.77	1575.77	17508.56	14.40
Error	21	1.88	0.09		

[1]All means are significantly different at $\alpha<0.001$.

agreement with Dawood (1979) who reported that the amount of myofibrillar proteins extracted from pre-rigor sucker muscle was higher than that from refrigerated sucker, and the latter was higher than that from freezer storage.

Effect of ice-storage on gel forming ability of sucker muscle.

Table 9 shows the changes in the relative concentration of myofibrillar proteins from ice-stored sucker flesh. Myosin heavy and light chains make up approximatly 60% of the total myofibrillar proteins . Actin is the second major myofibrillar protein, constituting 16% of the total. Total myosin concentration remained constant during the first four days of storage; it decreased slightly on the fifth day and dropped to 55% on the sixth day of storage. These results suggest that sucker muscle had passed post-mortem rigor, and possibly, gone through autolytic degradation. This could result in release of both C- and M-proteins from the thick filaments, perhaps increasing C- and M-protein extractability. Goll et al. (1977) explained that release of M-protein from M-zone situated in the center of the thick filaments causes the disruption of hexagonal lattice structure of the myofibrils. Consequently, a loss of the water holding ability and subsequent weakening of the gel forming ability would be expected.

According to Regenstein and Stamm (1979a,b), the water

Table 9. Changes in the relative percentage components of myofibrillar proteins[1] in sucker muscle during ice-storage from 0 to 6 days (SDS-PAGE analyses).

Components of myofibrillar proteins	Storage period, days						
	0	1	2	3	4	5	6
Myosin heavy chain	40.20	38.30	44.07	41.31	40.29	41.55	36.31
Myosin light chain[2]	11.75	11.93	8.94	11.97	11.05	9.01	10.19
Myosin light chain[3]	9.85	10.26	7.55	10.67	10.39	8.60	8.81
Total myosin	61.79	60.49	60.56	63.95	61.73	59.16	55.31
M-protein	4.01	3.19	4.06	3.42	2.94	4.66	5.50
C-protein	0.51	0.71	0.34	0.50	0.23	5.07	4.35
α-Actinin	1.69	1.68	1.59	1.40	0.92	1.31	1.27
Actin	16.77	17.10	21.32	16.72	18.43	15.18	17.08
β-tropomyosin	7.77	8.57	9.57	8.83	11.32	9.13	7.80
α-tropomyosin	7.26	8.27	2.79	5.20	4.45	5.49	7.26

[1]Averages represent duplicate determinations from two different batches.

[2]DTNB-light chain (DTNB-LC).

[3]Myosin light chain alkali A (LC-alkali A).

holding capacity (WHC) of raintrout white muscle and lobster tail muscle did not change from pre- to post-rigor. The trout muscle WHC values were similar to those of post-rigor chicken breast muscle and were not affected by the addition of pyrophosphate. In contrast, the WHC values of lobster muscle was like the WHC values of pre-rigor chicken breast muscle. The pre-rigor lobster muscle showed a large increase in WHC values with addition of pyrophosphate (205% of control). Their findings suggest that WHC for pre- and post-rigor muscle were species specific (Regenstein, 1977).

Results in Table 10 indicate that the gel strength of the cooked fish paste made from sucker flesh aged in ice from 0 to 4 days increased slightly, with a decrease beginning on the fifth day. However, the gel strength obtained during this period was not significantly different.

Table 10. Means and standard deviations of shear press values as an index for measurement of gel strength of fish balls made from fresh suckers stored in ice from 0 to 6 days.

Storage period, day	Pound shear force[1] per g sample
0	1.22±0.11
1	1.35±0.03
2	1.51±0.04
3	1.82±0.06
4	1.89±0.05
5	1.56±0.05
6	1.07±0.09

[1]Average of 3 determinations from each batch.

Data on the correlations between individual components of myofibrillar proteins and the gel strength of the cooked fish pastes are presented in Table 11. Results show that the gel strength of the heated fish pastes were directly related to the concentration of myosin, C-protein, actin, and β-tropomyosin in the salt soluble fraction of the sucker muscle. Myosin heavy or light chains alone were not strongly correlated with gel strength, whereas myosin heavy and light chains together had a significant effect on the gel strength. As indicated in Table 11, the r values for total myosin, myosin heavy chain, myosin light chain-DTNB and myosin light chain alkali A are 0.98, 0.55, 0.08, and 0.35 respectively. Mannherz and Goody (1976) reported that myosin heavy chain and myosin light chain alkali A are responsible for the binding of F-actin molecules while myosin light chain-DTNB is not responsible for myosin function. Myosin and actomyosin are considered to be the essential myofibrillar proteins that are needed for the best performance in gel formation and a firm product structure (Nakayama and Sato, 1971 a,b,c; Cheng et al., 1979). Also, at pre-rigor stage, more myosin and actin are bound together than in rigor and post-rigor stages. The high correlation of β-tropomyosin with gel strength (r=0.82) shown in this study is in agreement with the findings of Nakayama and Sato (1971c), and Cheng and Parrish (1979).

Table 11. The correlation between individual components of myofibrillar proteins and the gel strength of the cooked fish paste.

Components of myofibrillar proteins	Product-moment correlation, r[1]
Total myosin	0.98
Myosin heavy chain	0.55
Myosin light chain (DTNB-LC)	0.08
Myosin light chain (LC-Alkali A)	0.35
M-protein	-0.66
C-protein	0.56
α-Actinin	-0.53
Actin	0.14
β-tropomyosin	0.82
α-tropomyosin	-0.64

[1] $-1 \leq r \leq 1$, where upper limit implies that the values of myofibrillar proteins and gel strength of heated fish paste are directly related linearly in perfect harmony, the lower limit implies a perfect linear inverse relation, and zero implies no linear interdependence of the two variables.

Myosin heavy chain alone makes up approximatly 40% of the total sucker myofibrillar proteins, and contains all of the -SH groups of the myosin molecules. Itoh et al. (1980 a,b) proposed that the -SH groups are involved in the changes of native actomyosin property during heat treatment and gel formation. They found that the solubility of actomyosin decreased and the molecular weight of actomyosin increased during gel formation. They further suggested that the formation of the polymeric molecules of actomyosin could result from the formation of intermolecular disulfide bonds (-S-S-) in the heated actomyosin gel. Similarly, Buttkus (1971) proposed that intermolecular disulfide bonds are involved in the mechanism of protein denaturation.

The study on the effect of ice-storage on the myofibrillar protein extractability and gel forming ability lead to the conclusion that the myofibrillar proteins of the pre-rigor sucker flesh are more extractable and provide for better gel forming ability upon cooking than those in the rigor and post-rigor stages. After washing, the ratio of myofibrillar protein to sarcoplasmic proteins increased, resulting in greater gel forming capacity than that before washing.

In general, fish have a relatively shorter postmortem duration than the mammalian animals. The acceptable shelf life for ice-stored sucker usually does not exceed one week. This varies in different species and size of fish. After

harvesting, the fish should be processed as soon as possible. The location of the processing plant should be either on-board ship or near the unloading dock, so that the best quality of minced sucker flesh could be obtained before reaching the on-set of rigor stage. Prior to freezing, blending of minced sucker flesh with certain additives such as SHMP and MSG could also be a way of extending the quality stability of sucker flesh as a raw material for making fish paste products in later study.

The Effect of Washing on the Lipid Composition, Cholesterol Level and TBA Values of Mechanically Deboned Sucker Flesh

Data on the changes in lipid content, cholesterol level and TBA values in minced sucker flesh before and after washing are shown in Table 12. The washing technique removed primarily the unbound lipids and free cholesterol which had been released from the muscle tissues and bone marrow during the mechanical deboning process. As a result, a reduction in TBA values from 1.35 mg to 0.62 mg per kg of sucker flesh was realized. Total lipid content was decreased from 1.73g to 0.5g per 100g of sucker flesh. The cholesterol level was reduced from 44.90mg to 15.47mg per 100g of sucker flesh.

Data indicating the relative percentage of fatty acid composition in minced sucker flesh of both washed and unwashed samples are presented in Table 13. The results indicate that washing changes the relative percentage of fatty acid composition in total lipid as well as in its neutral lipid and phospholipid fractions.

Fatty acid content changes in total lipids after washing.

Changes in the relative percentage of total saturation and unsaturation in total lipid were negligible. Nevertheless, the relative concentration of mono- and dienoic

Table 12. The average total lipids, cholesterol and thiobarbituric acid (TBA) value[1] in minced sucker flesh before and after washing.

Batch	g lipid per 100 g samples		mg cholesterol per 100 g samples		TBA values, mg/kg sample	
	Before washing	After washing	Before washing	After washing	Before washing	After washing
Nov. 18, 1980	1.65±0.01	0.43±0.03	41.80±1.70	13.30±1.10	0.83±0.11	0.43±0.09
Nov. 22, 1980	1.67±0.07	0.44±0.02	42.40±2.60	15.70±2.30	1.42±0.09	0.66±0.04
April 15, 1981	1.87±0.03	0.63±0.02	50.51±0.40	17.40±1.00	1.80±0.10	0.77±0.03
Average	1.73±0.05	0.50±0.02	44.90±1.57	15.47±1.47	1.35±0.10	0.62±0.05

[1]The average were obtained from values of duplicate determinations.

acids in the total lipid decreased after washing, while the polyenoic acids increased. A decrease in mono-and dienoic acids contents was due to a slight reduction in fatty acids, C14:1, C15:1, C16:1, C17:1, C18:1, C20:1 and C18:2. An increase in the relative concentration of polyenoic acids resulted from a slight increase in fatty acids, C20:5, C22:5, C22:6 and a greater increase in C21:5.

Fatty acid content changes in neutral lipid through washing.

Washing has little effect on changes in fatty acid composition in neutral lipid of sucker flesh though the resulting data in Table 12 showed a great reduction in the total lipid content. While the total saturation in the neutral lipid fraction increased slightly after washing, the total unsaturation decreased. The relative concentrations of di- and polyenoic acids decreased slightly and changes in monoenoic acids were negligible. A slight increase in total saturation could be attributed to the slight increase in fatty acids, C16:0 and C18:0. A slight decrease in the relative total unsaturation could be due to a minor decrease in fatty acids, C20:1, C21:1, C18:2, C18:4 and C20:4. After washing, the concentration of all pentaenoic acids (C20:5, C21:5, C22:5) increased slightly and greater increase in hexaenoic acid (C22:6) also was found in neutral lipid fraction.

Table 13. Means and standard deviations of fatty acid composition[a] in minced sucker flesh before and after washing.

Fatty Acids	Neutral lipids		Phospholipids		Total lipids	
	Before Washing	After Washing	Before Washing	After Washing	Before Washing	After Washing
14:0	3.08±0.19	2.77±0.25	0.38±0.18	0.17±0.02	1.39±0.09	1.15±0.09
15:0	0.61±0.00	0.57±0.15	0.25±0.09	0.13±0.00	0.45±0.04	0.39±0.04
16:0	11.61±0.38	12.08±0.25	13.34±0.06	11.74±0.29	16.58±0.27	17.20±0.74
17:0	1.07±0.02	0.97±0.18	1.00±0.11	0.73±0.10	1.06±0.11	0.89±0.02
18:0	3.16±0.24	3.61±0.09	8.79±0.01	6.98±1.56	4.50±0.12	4.06±0.05
21:0	1.40±0.15	1.31±0.02	- ± -	0.07±0.06	0.66±0.05	0.61±0.18
14:1	1.53±0.06	1.42±0.01	- ± -	- ± -	0.63±0.07	0.44±0.10
15:1	0.66±0.05	0.63±0.09	0.32±0.32	0.81±0.05	0.45±0.04	0.29±0.03
16:1	24.66±1.34	24.22±0.30	5.05±0.77	3.53±0.02	12.32±0.80	11.12±0.25
17:1	2.07±0.23	2.03±0.10	0.63±0.15	0.69±0.07	1.22±0.04	1.03±0.03
18:1	20.71±0.25	21.13±0.14	9.28±0.49	8.64±0.71	12.77±0.41	11.98±0.08
20:1	1.49±0.00	1.88±0.10	0.33±0.10	0.41±0.10	0.91±0.11	0.76±0.03
21:1	3.81±1.33	3.34±0.09	8.48±0.34	7.25±0.61	6.59±0.28	6.72±0.02
24:1	0.26±0.07	0.55±0.03	1.73±0.20	1.47±0.13	1.19±0.27	1.58±0.10
18:2	5.41±0.50	4.61±0.30	1.53±0.16	1.67±0.00	3.24±0.38	2.49±0.02
20:2	0.38±0.09	1.02±0.37	0.46±0.07	1.77±1.30	0.29±0.09	0.52±0.16
18:3	0.47±0.01	0.56±0.01	- ± -	- ± -	0.58±0.31	0.20±0.04
18:4	2.02±0.05	0.18±0.02	0.64±0.05	1.70±1.13	1.45±0.06	1.13±0.14
20:4	2.21±0.02	0.38±0.08	0.50±0.07	0.57±0.01	0.63±0.36	0.53±0.11
20:5	6.92±0.35	7.57±0.37	10.13±0.07	8.47±0.67	10.18±0.10	11.16±0.37
21:5	0.27±0.23	0.70±0.38	0.45±0.17	9.43±0.78	0.72±0.15	2.10±0.13
22:5	2.19±0.17	2.71±0.18	5.93±0.07	5.48±0.40	4.20±0.05	4.43±0.10
22:6	3.98±0.48	5.77±0.56	30.75±0.44	28.26±1.20	18.01±0.46	19.21±1.32
% Sat. Fatty Acids	20.94±0.98	21.32±0.94	23.77±0.45	19.83±2.03	24.62±0.68	24.31±1.13
% Unsat. Fatty Acids	79.06±5.23	78.68±4.07	76.23±3.47	80.27±7.18	75.38±4.08	75.69±3.03
% Monoenoic Acids	55.20±3.33	55.20±0.86	25.84±2.37	22.82±1.69	36.07±2.02	33.92±0.64
% Dienoic Acids	5.79±0.59	5.62±0.67	1.99±0.23	3.54±1.30	3.53±0.47	3.01±0.18
% Polyenoic Acids	18.06±1.31	17.86±1.60	48.40±0.87	53.91±4.19	35.77±1.59	38.77±2.21

[a]Average of duplicate determinations from each batch of 250 lb sucker.

Fatty acid content changes in phospholipid through washing.

Washing of minced sucker flesh caused an increase in relative total unsaturation and a decrease in proportion of total saturation in the phospholipid fraction. The relative percentage of monoenoic acid declined as the amount of di-and polyenoic acids increased in phospholipids due to washing. The reduced relative concentration in monoenoic acid was due to a decrease in fatty acids, C16:1, C18:1, C21:1, and C24:1. An increase in the relative concentration of fatty acids, C18:2, C20:2, C18:4, C20:4 and C21:5 could be accounted for by an increase in the relative concentration of both di- and polyenoic acids. A decrease of docosahexaenoic acid (C22:6) from 30.75% to 28.26% after washing was probably due to the mechanical disruption of myofibrils resulting in the release of C22:6 acid from tissue phospholipids.

Washing the minced sucker flesh before processing and subsequent frozen storage is a practical way to improve quality and storage life. The washing technique removed not only sarcoplasmic constituents and blood materials, but also greatly reduced the unbound lipids, free cholesterol content and susceptibility to oxidation as indicated by TBA values. The fatty acid ratio of total unsaturation to saturation in total lipid increased slightly through washing, while in the phospholipid fraction, a shift from more saturation to less saturation through washing was observed.

Effect of Frozen Storage on the Lipid of Sucker Paste Products

Changes in quality of the frozen sucker paste products during storage were determined by analysis of fatty acid composition in total lipids, neutral lipids and phospholipids, analyses of phospholipid classes and TBA test as well as the sensory evaluation.

Changes due to lipid hydrolysis.

Data indicating the changes in relative percentage of neutral lipid and phospholipids of the sucker paste products are presented in Table 14. As the relative percentage of total phospholipids decreased, the total neutral lipid increased for all treatments. The magnitude of differences in the proportion of neutral lipids to phospholipids increased as the storage time increased. This evidence is further confirmed by the TLC analyses of phospholipid classes shown in Table 15 which suggests that decomposition of phosphatidyl ehtanolamine (PE) to lysophosphatidyl ethanolamine (LPE) began during the first month and continued upon further storage while phosphatidyl choline (PC) remained unchanged. This result is in agreement with Braddock (1970) who reported that the hydrolysis rate of PE to LPE in Coho salmon during 6 month frozen storage was greater than that of PC. In contrast, Bosund and Ganrot

Table 14. Means and standard deviations of the proportionate percentage changes in neutral lipids and phospholipids in sucker paste products during 6 months of frozen storage at -23°C.

Storage time, months	Raw Fish Paste								Cooked Fish Balls	
	NaCl-MSG-Sucrose		NaCl-MSG-SHMP		MSG-Sucrose-SHMP		NaCl-MSG-Sucrose-SHMP		NaCl-MSG-Sucrose-SHMP	
	Neutral lipid	Phospho-lipid	Neutral lipid	Phospho-lipid	Neutral lipid	Phospho-lipid	Neutral lipid	Phospho-lipid	Neutral lipid	Phospho-lipid
0	$46.05^{a}\pm1.30$	$53.95^{w}\pm1.33$	$45.81^{a}\pm1.97$	$54.19^{w}\pm2.03$	$45.54^{a}\pm0.27$	$54.46^{w}\pm0.32$	$44.74^{a}\pm0.86$	$55.26^{w}\pm0.86$	$39.55^{b}\pm0.22$	$60.45^{v}\pm0.15$
1	$50.49^{a}\pm1.02$	$49.51^{w}\pm0.98$	$47.76^{b}\pm1.05$	$52.24^{w}\pm1.05$	$46.15^{c}\pm0.40$	$53.85^{x}\pm0.40$	$46.12^{c}\pm0.75$	$53.88^{w}\pm0.75$	$40.04^{d}\pm0.27$	$59.96^{v}\pm0.59$
2	$51.20^{a}\pm0.75$	$48.80^{w}\pm0.73$	$48.30^{b}\pm1.07$	$51.70^{w}\pm1.06$	$48.05^{b}\pm1.30$	$51.95^{w}\pm1.30$	$49.50^{b}\pm0.20$	$50.50^{w}\pm0.20$	$40.46^{c}\pm0.67$	$59.54^{v}\pm0.70$
3	$57.04^{a}\pm0.64$	$42.96^{x}\pm0.60$	$49.57^{b}\pm0.94$	$50.43^{w}\pm0.92$	$49.51^{b}\pm0.74$	$50.49^{w}\pm1.23$	$51.24^{b}\pm0.19$	$48.76^{w}\pm0.19$	$41.35^{c}\pm0.17$	$58.65^{v}\pm0.14$
4	$58.38^{a}\pm0.13$	$41.62^{z}\pm0.13$	$50.14^{c}\pm0.46$	$49.86^{x}\pm0.46$	$50.00^{d}\pm0.50$	$50.00^{w}\pm0.50$	$52.96^{b}\pm0.16$	$47.04^{y}\pm0.16$	$42.33^{e}\pm0.20$	$57.67^{v}\pm0.20$
5	$57.88^{a}\pm0.71$	$42.12^{z}\pm0.71$	$51.06^{c}\pm1.60$	$48.94^{x}\pm1.44$	$50.47^{d}\pm0.31$	$49.53^{w}\pm0.31$	$53.32^{b}\pm0.27$	$46.68^{y}\pm0.27$	$43.26^{e}\pm0.39$	$56.74^{v}\pm0.37$
6	$58.72^{a}\pm0.67$	$41.28^{y}\pm0.84$	$54.09^{b}\pm0.93$	$45.91^{x}\pm0.68$	$51.44^{c}\pm0.18$	$48.56^{w}\pm0.18$	$54.01^{b}\pm0.47$	$45.99^{x}\pm0.47$	$45.86^{d}\pm0.72$	$54.14^{v}\pm0.75$

[1]Average of two determination from each batch.

^{abcde}Significant differences among treatments at $\alpha<0.01$ with different superscripts for neutral lipids.

^{vwxyz}Significant differences among treatments at $\alpha<0.01$ with different superscripts for phospholipids.

Table 15. Qualitative changes of phospholipid classes by TLC analyses

Treatment	Phospholipid Classes	Storage period, months					
		1	2	3	4	5	6
Raw Fish Pastes:							
NaCl-MSG-Sucrose	PE	X	X	X	X	X	X
	PC	X	X	X	X	X	X
	LPE	X	X	X	X	X	X
	LPC						
NaCl-MSG-SHMP	PE	X	X	X	X	X	X
	PC	X	X	X	X	X	X
	LPE	X	X	X	X	X	X
	LPC						
MSG-MSG-SHMP	PE	X	X	X	X	X	X
	PC	X	X	X	X	X	X
	LPE	X	X	X	X	X	X
	LPC						
NaCl-MSG-Sucrose-SHMP	PE	X	X	X	X	X	X
	PC	X	X	X	X	X	X
	LPE	X	X	X	X	X	X
	LPC						
Cooked Fish Balls	PE	X	X	X	X	X	X
(NaCl-MSG-Sucrose-SHMP)	PC	X	X	X	X	X	X
	LPE	X	X	X	X	X	X
	LPC						

PE = Phosphatidyl ethanolamine.
PC = Phosphatidyl choline.
LPE = Lyso-phosphatidyl ethanolamine.
LCP = Lyso-phosphatidyl choline.
X = Presence of a specific phospholipids.

(1969 a,b) showed that in frozen stored Baltic herring, the hydrolysis rate of PC was faster than that of PE in both white and dark muscle. This suggests that the rate of hydrolysis of PE and PC to their lyso-derivatives differs in various species.

The decomposition of PE to its lyso-derivative in raw fish pastes could be attributed to enzymatic lipolysis while the decomposition of PE occurring in cooked fish balls was not totally enzymatic lipolysis. Between the time of processing and cooking, the change in PE was brought about by emzymatic lipolysis. After cooking, the tissue enzyme system was inactivated; thus it is unlikely that further lipolytic decomposition of PE to LPE occurred during frozen storage. Nevertheless, the presence of LPE in the cooked fish balls was continually monitored by TLC analyses during 6 months of frozen storage. Presumably, this LPE originated from lipolysis of PE before cooking. The hypothesis is that hydrolysis of PE to LPE indicates a decrease in the relative phospholipid content of raw fish pastes due to enzymatic action; this should result in free fatty acid (FFA) production and formation of lyso-derivatives. The FFA release from PE could be a factor contributing to further oxidation and protein denaturation (Roubal, 1967; Awad et al., 1969; Braddock and Dugan, 1969; Shenouda, 1980). TBA values in this study, however, remained low during the 6 months of storage. Buttkus

(1967) has postulated that a reaction between myosin and malonaldehyde may occur during storage and caused a decline in TBA value.

The following explains why there was no apparent PC decomposition: (1) low phospholipase activity or lysolecithinase may be active, (2) PC, per se, functions as a natural emulsifier which prevents separation of PC from fish muscle protein, and (3) SHMP is known to have emulsifying ability in maintaining a lipid-protein-water emulsion in which phosphates prevent the protein from denaturing, thus maintaining the hydrated form of protein and preventing PC-protein separation in the sucker paste system.

Comparison of the relative percentage of fatty acids of phospholipids in the fresh minced sucker flesh and frozen stored sucker pastes is shown in Table 16. The changes in fatty acid contents in the phospholipid fraction mainly occurred between the initial time of processing and the first month of frozen storage. Upon further storage, the changes were small suggesting that changes during frozen storage were minor. The primary change occurring between 0 time and one month of storage could be caused by the prolonged exposure of raw samples to the open atmosphere at room temperature during processing that promoted lipid autoxidation, and /or lipolysis of raw fish pastes.

Table 16. Changes in proportionate percentage of the selected fatty acids in the phospholipids of fish pastes and cooked fish balls during 6 months of frozen storage at -23°C.

Fatty Acids	Significant differences at $\alpha<0.05$		Percent fatty acids, at initial	Raw Fish Pastes												Cooked Fish Balls		
				#1 NaCl-MSG-Sucrose			#2 NaCl-MSG-SHMP			#3 MSG-Sucrose-SHMP			#4 NaCl-MSG-Sucrose-SHMP			NaCl-MSG-Sucrose-SHMP		
	Treatment	Month	0*	1*	3*	6*	1	3	6	1	3	6	1	3	6	1	3	6
14:0	T		0.17	0.45	0.44	0.65	0.41	0.58	0.61	0.64	0.32	0.59	0.55	0.44	0.55	0.43	0.41	0.46
15:0		M	0.13	0.24	0.25	0.43	0.10	0.26	0.40	0.38	0.21	0.40	0.36	0.29	0.42	0.28	0.22	0.34
16:0		M	11.74	18.77	18.78	20.12	18.94	19.13	19.80	19.56	19.32	20.34	19.13	19.51	20.85	17.60	18.14	20.22
18:0			6.98	5.18	4.75	4.59	4.90	4.61	5.35	4.30	5.43	5.80	5.31	5.70	5.10	6.34	5.13	4.39
14:1		M	0.00	0.00	0.00	0.15	0.00	0.00	0.15	0.15	0.00	0.11	0.12	0.03	0.10	0.07	0.04	0.03
16:1			3.53	5.41	5.22	5.46	5.48	4.76	5.61	7.29	4.86	5.24	5.66	5.11	5.66	4.98	5.31	5.70
17:1		M	0.69	0.62	0.60	0.57	0.55	0.55	0.70	0.79	0.39	0.46	0.70	0.61	0.45	0.58	0.46	0.49
18:1		M	8.64	8.58	8.40	9.87	8.56	9.10	9.90	10.75	8.35	9.30	9.47	8.66	9.71	9.02	9.23	10.02
21:1			7.25	8.93	8.41	8.49	9.12	8.40	8.28	8.06	8.58	8.61	8.25	8.62	8.57	8.72	8.67	8.33
24:1		M	1.47	1.78	2.32	1.24	2.22	1.95	1.44	1.20	2.15	1.34	1.47	1.76	1.27	1.33	1.69	1.45
20:5			8.47	12.75	11.46	11.14	12.04	11.70	11.03	11.25	11.24	11.06	11.69	11.24	11.22	11.08	11.68	11.58
21:5		M	9.43	1.79	3.73	0.48	2.83	2.79	0.77	0.58	3.72	0.53	1.16	2.07	0.40	0.60	1.49	1.06
22:5		M	5.48	5.19	5.38	4.99	5.25	5.30	5.09	4.64	5.45	5.10	4.95	5.10	5.08	5.20	5.39	5.20
22:6		M	28.26	25.06	25.60	25.48	24.42	25.98	24.73	23.50	25.97	26.18	24.54	25.99	25.62	26.24	26.87	25.43

*0 = Initial percentage of fatty acids in phospholipids of washed sample containing no additives.
1 = At the end of first month of storage.
3 = At the end of third month of storage.
6 = At the end of sixth month of storage.
T = Treatment.
M = Month of storage.

Analysis of fatty acid changes immediately after processing were not conducted in this study. Therefore, it is not possible to state whether changes of fatty acid contents were caused by processing or by the initial slow rate of freezing. A further study is needed to confirm fatty acid changes in sucker paste products caused by processing per se.

The relative percentage of fatty acids, C16:0, C16:1, and C20:5 in raw fish pastes generally increased from the initial value to one month of storage while the relative percentage of C18:0, C21:5 and C22:6 acids decreased. In cooked fish balls , the relative percentage of palmitic acid(C16:0) increased greatly from an initial 11.74% to 17.60% during the first month and after that, the rate of increase in C16:0 acid was less pronounced. A slight increase in the relative concentration of fatty acids C16:1, C18:1 and C20:5 was also observed in cooked fish balls between the initial value and one month of frozen storage. The relative concentration of C21:5 and C22:6 acids in both raw and cooked fish pastes decreased significantly from initial to one month of storage. The changes reflected losses of specific fatty acids from phospholipid fraction, which could be caused by enzymatic lipolysis and interconversion of longer chained to shorter chained acids (Hardy et al., 1979). A significant increase in the relative percentage of palmitic acid (C16:0) at an early stage of

freezing and prior to freezing could possibly be resulted from hydrolysis of PE to LPE and release of C16:0 acid as mentioned previously and from hydrolysis of C21:5 and C22:6 acids.

According to Mai and Kinsella (1979b), the major components of FFA found in white sucker flesh were palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), arachidonic acid (20:4), eicosapentaenoic acid (C20:5), and docosahexaenoic acid (C22:6). This evidence is consistant with the finding of this investigation.

Braddock (1970) noted a preferential hydrolysis of PE in Coho salmon containing C16:0 and C22:6 acids. The release of C16:0 acid from phosphoglyceride molecules through phospholipase action preferentially occurred in the Sn-2 position. It is also known that polyunsaturated fatty acids, C16:0 and short chain fatty acids of fish accumulate mainly at the Sn-2 position of a triglyceride and /or phosphoglyceride molecules (Brockerhoff et al., 1968; Braddock and Dugan, 1972; Dugan, 1976). Whether the C16:0 acid found in phospholipids of sucker flesh is actually accumulated at the Sn-2 position needs further study. The rate of enzymatic hydrolysis of lipid is pH and temperature dependent, and may be retarded by the hydrolysis end product concentration. The freezing temperature fluctuation observed in the present study could be one of the factors that caused a slight change in quality of sucker paste products.

Oxidative changes.

The changes in fatty acid composition in total lipids, neutral lipids and phospholipids of the frozen sucker paste products used in the present study are shown in Table 16 to 21 and Appendix B1 to B16. The primary oxidative changes in fatty acids probably occurred during processing and early stage of frozen storage as mentioned previously in this study. During the first month of storage, the changes in the relative percentage of total saturated fatty acids in the total lipids of the raw fish pastes were slight while the relative percentage of the total unsaturated fatty acids tended to decrease. This decrease was primarily due to a decrease in polyenoic acids. Cooked fish balls showed a slight increase in total unsaturated fatty acids and a decrease in the total saturated fatty acids during the first month of frozen storage. After 6 months, the relative percentage of total saturated fatty acids in total lipids increased slightly while the relative percentage of total unsaturated fatty acids tended to decrease. The lower dienoic acid content reflected to the decrease in total unsaturated fatty acids for both raw and cooked samples after 6 months of storage. However, the raw fish paste that contained MSG-Sucrose-SHMP showed a reverse trend, a slight increase in total unsaturated fatty acids and a slight decrease in total saturated fatty acids. This finding suggests that treatmnet with MSG-Sucrose-SHMP

Table 17. Changes in proportionate percentage of fatty acid composition of total lipids in sucker paste products during 6 months of frozen storage.

Fatty Acid Classes	Month of storage at -23°C			
	0[1]	1	3	6
% saturated fatty acids	24.31±1.13			
Raw Fish Pastes				
NaCl-MSG-Sucrose		26.41±1.03	25.13±2.81	25.77±1.06
NaCl-MSG-SHMP		26.08±2.08	26.22±0.72	26.97±1.56
MSG-Sucrose-SHMP		26.02±1.30	24.52±0.91	23.91±0.41
NaCl-MSG-Sucrose-SHMP		26.56±3.89	25.72±1.35	25.93±1.07
Cooked Fish Balls		23.03±2.02	26.04±0.34	25.26±0.64
% unsaturated fatty acids	75.69±3.03			
Raw Fish Paste				
NaCl-MSG-Sucrose		73.38±3.32	73.23±12.84	74.31±1.92
NaCl-MSG-SHMP		73.97±7.51	74.06±5.25	73.44±2.11
MSG-Sucrose-SHMP		73.91±10.79	75.53±10.26	76.51±1.63
NaCl-MSG-Sucrose-SHMP		73.01±13.24	74.38±6.94	74.02±1.70
Cooked Fish Balls		77.06±4.92	74.52±1.92	74.99±2.84
% Monoenoic acids	33.92±0.64			
Raw Fish Pastes				
NaCl-MSG-Sucrose		32.48±1.55	36.54±6.27	34.25±0.80
NaCl-MSG-SHMP		33.93±3.93	33.67±3.40	32.31±0.59
MSG-Sucrose-SHMP		35.95±5.27	36.39±5.21	35.13±1.35
NaCl-MSG-Sucrose-SHMP		35.42±6.93	37.83±4.08	32.04±0.84
Cooked Fish Balls		34.07±2.70	32.16±1.59	32.51±1.67
% Dienoic acids	3.01±0.18			
Raw Fish Pastes				
NaCl-MSG-Sucrose		2.47^{b}±0.34	1.51^{c}±0.81	3.18^{a}±0.33
NaCl-MSG-SHMP		2.72 ±0.20	2.45 ±0.15	2.79 ±0.22
MSG-Sucrose-SHMP		3.06 ±0.32	3.20 ±0.40	3.17 ±0.28
NaCl-MSG-Sucrose-SHMP		2.82_{a}±0.82	2.86_{c}±0.29	2.77_{b}±0.43
Cooked Fish Balls		3.26^{a}±0.32	2.44^{c}±0.14	2.96^{b}±0.22
% Polyenoic acids	38.77±2.21			
Raw Fish Pastes				
NaCl-MSG-Sucrose		38.43±1.43	35.18±5.76	36.88±0.79
NaCl-MSG-SHMP		37.32±3.38	37.94±1.70	37.34±1.30
MSG-Sucrose-SHMP		34.90±5.20	35,94±4.65	38.21±1.76
NaCl-MSG-Sucrose-SHMP		34.77±5.49	33.69±2.57	39.21±0.43
Cooked Fish Balls		39.73±1.90	39.92±0.19	39.52±0.95

^{abc}Figures at the horizontal line with different superscripts have significant difference at $\alpha<0.05$ during storage time (Turkey's test of statistics).

1 0=Initial level of fatty acids without adding additives.

2 Analyses of variance were not significant.

3 Analyses of variance established a significant difference among time ($\alpha<0.05$).

renders the paste more stable to lipid oxidation. In this case, the prevention of polyunsaturated fatty acid(PUFA) oxidation could be attributed to the metal chelating effect of SHMP. The polyenoic acid content of the total lipid decreased slightly but not significantly in raw fish pastes containing NaCl-MSG-Sucrose and NaCl-MSG-SHMP, while little change in the polyenoic acid content of cooked fish balls and raw fish pastes containing MSG-Sucrose-SHMP and NaCl-MSG-Sucrose-SHMP were found after six months of storage at -23^{o}C. Changes in the relative percentage of some major fatty acids of the total lipids (Table18) were not significant among treatments throughout six months of frozen storage period except linoleic acid (C18:2) which fluctuated in the raw fish pastes containing NaCl-MSG-Sucrose. The fluctuation may be related to the oxidative products resulting in the formation of TBA-malonaldehyde complex and oxidative products other than malonaldehyde which reacted slowly with the TBA reagent to yield a yellow compound (Yu and Sinnhuber, 1962). Linoleic acid is also known to interact with myosin in frozen muscle resulting in muscle protein insolubilization (King et al., 1962; Braddock, 1970).

The rate at which the relative percentage of total neutral lipids increased and phospholipid decreased was faster in raw fish pastes than that in the cooked fish balls; and among raw fish pastes the rate of change is

Table 18. Changes in the proportionate percentage of selected fatty acids in total lipids of sucker paste products during 6 months of storage at -23°C.

Fatty Acids	Significant difference at $\alpha<0.05$ Treatment Month	Percent fatty acids, initial at 0 time	Treatments														
			Raw												Cooked		
			NaCl-MSG-Sucrose			NaCl-MSH-SHMP			MSG-Sucrose-SHMP			NaCl-MSG-Sucrose-SHMP			NaCl-MSG-Sucrose-SHMP		
			1	3	6	1	3	6	1	3	6	1	3	6	1	3	6
16:0		17.16	18.86	18.28	17.95	18.54	19.07	18.89	17.59	16.54	16.72	18.91	17.72	18.63	15.77	19.34	17.91
18:0		4.05	4.95	4.18	4.80	4.75	4.48	5.34	4.95	4.98	4.43	4.59	4.45	4.65	4.41	4.40	4.66
16:1		11.09	9.96	12.60	10.49	10.95	11.20	9.85	12.17	12.01	11.58	12.79	13.85	9.69	10.64	9.85	9.86
18:1		11.95	11.59	13.39	12.56	11.96	11.99	11.96	13.03	13.30	12.70	13.40	13.57	11.48	11.98	11.29	11.70
21:1		6.70	7.47	6.91	7.46	7.40	7.39	7.24	6.60	6.89	7.22	6.62	6.35	7.65	7.78	7.93	7.42
18:2	M	2.49	2.25	1.12	2.68	2.32	2.08	2.38	2.69	2.71	2.78	2.54	2.49	2.29	2.73	2.06	2.56
20:5		11.13	10.74	10.24	10.25	10.76	10.98	10.37	10.06	10.24	10.86	10.50	10.02	11.07	11.02	11.40	11.06
22:5		4.42	4.63	4.19	4.48	4.51	4.54	4.66	4.12	4.49	4.44	4.22	4.14	4.62	4.60	4.68	4.72
22:6		19.17	21.05	18.40	19.53	20.12	20.63	20.42	18.12	18.80	20.01	18.45	17.26	21.50	20.72	22.17	21.20

[1]At the end of the first month of storage.

[3]At the end of the third month of storage.

[6]At the end of the sixth month of storage.

[M]Month

more pronounced in samples containing NaCl-MSG-Sucrose, and followed by NaCl-MSG-SHMP, NaCl-MSG-Sucrose-SHMP and MSG-Sucrose-SHMP in decreasing order. Thus, fish pastes with NaCl added but without SHMP showed a greater decrease in phospholipids than pastes containing SHMP but no NaCl. Data on the changes in relative percentage of fatty acid composition in neutral lipids in sucker paste products are presented in Table 19 and 20. Although changes in the relative percentage of some fatty acids in neutral lipids were found due to treatment and length of storage, the changes were relatively small. Also, the neutral lipids of sucker flesh contained 21% oleic acids, which has been found to have a strong inhibitory effect on the phospholipase B activity of cod muscle (Yorkowski and Brockerhoff, 1965). If this is true for the sucker pastes, this may explain why only minor changes in the fatty acid composition of the neutral lipids were detected during six months of frozen storage. In addition, polyunsaturated fatty acid in phospholipids of the fish oxidize before substantial oxidation Of the triglyceride occurs (Dugan, 1976). However, evidence showed that free fatty acid production during frozen storage may occur in certain species through hydrolysis of triglycerides (Bosund and Ganrot, 1969).

Data on changes in fatty acid concentration in phospholipids were rapid during the first month of storage and /or prior to freezing; whereas upon storage, the changes

that occurred in the fatty acid of phospholipid was relatively small. The oxidation that occurred prior to and during the first month of storage could be attributed to the mechanical action of mincing which caused disruption of muscle membranes, liberating fatty acids from tissue phospholipids, increasing surface area of tissue muscle to be exposed to atmosphere at room temperature, thereby, enhancing lipid autoxidation and /or hydrolysis, and lipid-protein interaction. It is also known that trace amount of sarcoplasmic enzymes and heme compounds could accelerate hydrolytic and oxidative degradation in minced muscle food systems respectively (Lee and Toledo, 1976; Schnell et al., 1973; Igene et al., 1979). Substances were present in the sucker paste system which affect the oxidation rate. SHMP acts as a metal sequestrant for iron which catalyzes oxidative rancidity. Sodium chloride which was used to extract muscle proteins, at the same time can have a prooxidant effect on promoting lipid perooxide formation. Thus, the chemical inhibition of lipid oxidation by SHMP was involved in the later months of storage. The overall decline in the relative percentage of total unsaturated fatty acids in phospholipids of sucker paste products was found mainly at the stage prior to and during the first month of storage. The decrease in dienoic and polyenoic acids could be due both to hydrolytic and oxidative changes prior to freezing storage. The relative concentration of total saturated

Table 19. Changes in proportionate percentage of fatty acid composition of neutral lipids in sucker pastes during 6 months of storage at -23°C.

Fatty acid classes	Significant differences at α<0.05 Treatment	Month	Percent fatty acids at initial, 0 time	Storage Period, Months 1	2	3	4	5	6
% Sat. Fatty Acids	T		21.32±0.95						
Raw fish pastes									
NaCl-MSG-Sucrose				20.96±0.67	20.73±0.40	20.96±0.54	21.05±0.24	21.29±0.76	20.65±0.38
NaCl-MSG-SHMP				21.01±0.24	21.15±0.49	21.35±0.50	21.09±1.08	21.88±0.68	21.11±0.61
MSG-Sucrose-SHMP				22.06±0.70	21.44±0.71	21.80±0.28	22.19±1.39	22.17±1.59	21.83±0.56
NaCl-MSG-Sucrose-SHMP				20.86±0.65	20.42±0.50	21.37±1.17	21.60±1.57	20.75±1.32	21.42±0.52
Cooked fish balls				21.84±0.87	21.28±0.47	21.00±0.59	20.56±0.74	21.16±0.77	21.27±0.18
% Unsat. Fatty Acids	T		78.68±4.07						
Raw fish pastes									
NaCl-MSG-Sucrose				79.58±2.28	79.20±2.45	79.26±2.14	79.02±2.86	79.50±2.92	79.08±4.12
NaCl-MSG-SHMP				78.94±2.34	78.98±2.44	79.22±1.83	78.97±2.54	78.06±1.86	80.79±1.52
MSG-Sucrose-SHMP				77.95±3.99	79.49±6.58	78.00±6.35	77.90±6.89	77.34±4.78	78.25±2.44
NaCl-MSG-Sucrose-SHMP				78.69±3.68	79.51±2.21	78.69±2.75	78.42±3.68	79.30±3.77	78.81±2.43
Cooked fish balls				78.26±2.70	78.77±2.95	78.98±2.37	79.45±1.54	78.70±4.39	78.66±3.13
% Monoenoic Acids	T		55.20±0.86						
Raw fish pastes									
NaCl-MSG-Sucrose				54.93±1.28	54.92±1.67	55.30±1.07	54.25±0.86	54.94±1.39	54.84±2.47
NaCl-MSG-SHMP				54.14±0.88	53.33±1.96	53.74±0.83	52.61±1.33	53.25±1.15	53.83±0.54
MSG-Sucrose-SHMP				53.06±2.76	54.38±3.79	51.26±2.78	54.14±3.47	52.19±2.11	51.21±0.73
NaCl-MSG-Sucrose-SHMP				53.90±2.09	53.99±0.97	51.94±1.64	54.42±2.28	53.20±1.68	53.23±1.02
Cooked fish balls				52.68±1.36	53.91±1.49	54.91±1.37	54.76±1.12	54.16±2.14	54.97±1.89
% Dienoic Acids	T		5.62±0.67						
Raw fish pastes									
NaCl-MSG-Sucrose				5.69±0.41	4.97±0.07	4.91±0.33	4.92±0.23	5.17±0.23	5.05±0.54
NaCl-MSG-SHMP				5.51±0.74	5.18±0.47	5.26±0.27	5.64±0.46	4.87±0.28	5.29±0.47
MSG-Sucrose-SHMP				4.69±0.15	5.26±0.59	4.49±0.33	4.07±0.35	4.76±0.09	5.12±0.65
NaCl-MSG-Sucrose-SHMP				4.83±0.38	5.02±0.35	4.97±0.06	4.91±0.10	5.02±0.32	5.51±0.36
Cooked fish balls				5.44±0.38	5.31±0.41	5.31±0.24	5.39±0.19	5.86±0.78	5.42±0.23
% Polyenoic acids	T		17.86±1.60						
Raw fish pastes									
NaCl-MSG-Sucrose				18.96±0.59	19.31±0.71	19.05±0.74	19.85±1.77	19.39±1.30	19.19±1.11
NaCl-MSG-SHMP				19.29±0.72	20.47±0.61	20.22±0.73	20.72±0.75	19.97±0.43	21.67±0.51
MSG-Sucrose-SHMP				20.20±1.08	19.85±2.20	22.25±3.24	19.69±3.07	20.39±0.99	21.92±1.06
NaCl-MSG-Sucrose-SHMP				19.96±1.21	20.50±0.89	21.78±1.05	19.09±1.30	21.08±1.77	20.07±1.05
Cooked fish balls				20.14±0.96	19.55±1.05	18.76±0.76	19.30±0.23	18.68±1.47	18.27±1.01

T=Treatment

Table 20. Changes of the proportionate percentage of the selected fatty acids in the neutral lipids of raw sucker pastes and cooked fish balls during 6 months of storage at -23°C.

Fatty Acids	Significant differences at $\alpha<0.05$		Percent fatty acid, initial	Storage period, months														
				1st month					3rd month					6th month				
	Treatment	Month	0	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5
14:0	T		2.77	2.77	2.70	2.54	2.72	2.66	2.75	2.81	2.28	2.62	2.81	2.63	2.64	2.35	2.56	2.86
15:0		M	0.57	0.53	0.52	0.56	0.59	0.59	0.48	0.50	0.43	0.41	0.54	0.52	0.59	0.55	0.58	0.56
16:0	T	M	12.08	12.03	11.96	12.66	12.26	12.68	12.43	12.70	13.50	13.02	12.30	11.99	12.13	12.87	12.26	12.36
17:0		M	0.97	1.06	1.03	1.09	0.79	1.07	0.85	0.57	0.65	0.57	0.87	1.01	1.13	1.10	1.14	1.04
18:0	T		3.61	3.32	3.26	3.82	3.28	3.62	3.32	3.49	3.68	3.55	3.30	3.32	3.35	3.85	3.51	3.30
14:1	T	M	1.42	1.36	1.41	1.31	1.42	1.40	1.42	1.20	0.66	1.08	1.45	1.37	1.44	1.20	1.53	1.46
15:1	T	M	0.63	0.59	0.57	0.62	0.59	0.63	0.54	0.51	0.37	0.46	0.56	0.52	0.68	0.62	0.68	0.55
16:1	T		24.22	24.46	23.62	22.39	23.45	23.41	24.74	23.81	21.91	22.90	24.86	23.37	23.13	20.84	22.28	24.49
18:1	T		21.13	21.24	20.72	20.18	20.55	20.05	21.08	20.70	19.24	19.96	21.05	21.59	20.28	19.81	20.21	21.16
21:1	T		1.88	3.47	3.60	4.31	3.79	3.42	3.34	3.76	4.04	4.00	3.01	4.12	3.91	4.56	3.97	3.28
24:1	T		0.55	0.49	0.36	0.42	0.47	0.48	0.57	0.44	1.97	0.54	0.40	0.50	0.58	0.55	0.67	0.36
18:2	T		4.61	5.08	4.82	4.32	4.38	4.77	4.58	4.85	4.00	4.66	4.99	4.48	4.63	4.42	4.60	4.98
20:2		M	1.02	0.61	0.69	0.37	0.45	0.67	0.33	0.41	0.49	0.31	0.32	0.57	0.66	0.70	0.91	0.44
18:3		M	0.56	0.49	0.61	0.44	0.42	0.46	0.49	0.49	0.40	0.36	0.51	0.44	0.57	0.52	0.63	0.49
18:4	T	M	0.18	1.93	2.20	1.91	1.92	2.00	2.00	1.93	1.82	1.71	2.00	1.92	1.98	1.92	2.12	2.00
22:6	T		5.77	5.33	5.60	6.41	6.05	6.17	5.93	6.00	6.75	7.64	5.36	5.69	6.10	7.79	6.28	5.22

0 = Initial percentage of fatty acids in neutral lipids of washed minced sucker flesh containing no additives.
#1 = Raw fish paste added with NaCl, MSG and sucrose.
#2 = Raw fish paste added with NaCl, MSG and SHMP.
#3 = Raw fish paste added with MSG, sucrose and SHMP.
#4 = Raw fish paste added with NaCl, MSG, sucrose and SHMP.
#5 = Cooked fish balls added with NaCl, MSG, sucrose and SHMP.
T = Treatment
M = Month

fatty acids and monoenoic acids increased rapidly from 0 to one month of storage but only increased slightly upon further storage (Table 21). This suggests that further lipolytic degradation of phospholipids may have been retarded by either low temperature or its hydrolysis end products after the first month of storage. A further minor change in phospholipid after the first month of storage could be attributed to autoxidation which might have been promoted by NaCl. A slow rate of increase in TBA values in this study indicates that slight autoxidation may have occurred during frozen storage.

Production of malonaldehyde in frozen fish paste products in this study was relatively low (Table 22). During six months of storage at -23^{o}C, the TBA values of raw fish pastes containing NaCl-MSG-Sucrose, NaCl-MSG-SHMP, MSG-Sucrose-SHMP, and NaCl-MSG-Sucrose-SHMP ranged from 0.58 to 1.36, 0.33 to 0.67, 0.24 to 0.49, and 0.26 to 0.65 respectively; while the TBA values for the cooked fish balls ranged from 0.04 to 0.47. These TBA values could be correlated with the acceptable quality of fish since they were below TBA values of 2 (Yu and Sinnhuber, 1957). Evidence showed that raw fish pastes except those with no NaCl added had higher TBA values than cooked fish balls. Among raw fish pastes, samples containing NaCl with or without SHMP had higher TBA values than those containing only SHMP. This result suggests that SHMP may have a preventive effect on

Table 21. Changes in proportionate percentage of fatty acid composition of phospholipids in sucker paste products during 6 months of storage at -23°C.

Fatty Acid Classes	Significant Difference at $\alpha<0.05$ T	M	Initial Fatty Acid, % at 0 time*	Months of Storage at -23°C 1	2	3	4	5	6
% Sat. Fatty Acids	T	M	19.83±2.03						
Raw Fish Pastes									
#1				25.49±0.65	26.19±0.48	25.03±1.31	25.43±0.75	26.58±0.25	26.73±1.04
#2				25.20±0.57	25.91±1.11	25.29±1.19	26.83±0.96	27.71±1.52	27.12±0.63
#3				27.11±3.41	26.35±0.56	26.01±0.71	26.01±1.66	26.72±1.53	28.02±1.04
#4				26.50±1.27	26.87±0.72	26.83±0.52	26.46±0.24	27.87±1.62	27.93±1.24
Cooked Fish Balls				25.55±3.04	25.90±0.54	24.69±0.87	26.26±0.69	26.66±0.13	26.25±0.11
% Unsat. Fatty Acids	T	M	80.27±7.18						
Raw Fish Pastes									
#1				74.56±3.83	74.87±2.42	75.02±1.99	74.54±2.62	73.15±2.52	73.24±3.07
#2				74.86±1.82	74.08±5.45	74.77±3.15	73.24±1.67	72.28±1.61	72.49±2.31
#3				72.92±4.40	74.44±1.79	74.48±2.62	74.03±2.13	74.17±3.03	72.01±2.25
#4				73.55±2.46	73.67±3.09	73.26±3.12	73.56±1.94	72.12±2.99	72.20±1.89
Cooked Fish Balls				74.57±4.31	74.13±1.21	75.70±2.90	73.96±4.21	73.49±2.67	73.58±1.95
% Monoenoic Acids		M	22.82±1.69						
Raw Fish Pastes									
#1				26.40±0.88	27.80±0.89	25.92±0.54	26.82±0.77	28.47±0.67	27.02±0.96
#2				26.73±0.62	26.87±3.21	25.82±1.23	26.92±0.84	26.69±0.74	27.07±1.00
#3				29.36±2.16	26.78±1.31	25.16±1.17	26.66±1.05	25.95±1.49	25.96±0.74
#4				26.93±0.80	26.52±1.50	25.83±0.75	26.41±0.81	26.20±0.85	26.62±0.92
Cooked Fish Balls				26.02±1.05	25.22±0.49	26.74±0.89	26.19±1.66	26.68±0.82	26.89±1.04
% Dienoic Acids	T		3.54±1.30						
Raw Fish Pastes									
#1				2.17±0.30	2.50±0.40	1.96±0.13	2.79±0.27	2.94±0.74	2.73±0.36
#2				2.20±0.06	2.07±0.26	2.14±0.06	2.39±0.16	2.19±0.31	2.03±0.17
#3				2.27±0.63	2.01±0.07	2.00±0.41	2.25±0.19	1.91±0.01	2.04±0.53
#4				2.69±0.27	2.44±0.26	2.07±0.20	1.84±0.04	2.04±0.10	2.13±0.39
Cooked Fish Balls				3.10±0.50	2.18±0.13	2.39±0.19	2.17±0.37	2.14±0.03	2.20±0.08
% Polyenoic Acids		M	53.91±4.19						
Raw Fish Pastes									
#1				45.99±2.65	44.57±1.13	47.14±1.32	44.93±1.58	43.90±1.11	43.49±1.75
#2				45.93±1.14	45.14±1.98	46.81±1.86	43.93±0.67	43.40±0.66	43.39±1.14
#3				41.29±1.61	45.65±0.41	47.32±1.04	45.12±0.89	46.31±1.53	44.01±0.98
#4				43.93±1.39	44.71±1.33	45.46±2.17	45.13±1.09	43.38±2.04	43.45±0.58
Cooked Fish Balls				45.45±2.76	46.73±0.59	46.57±1.85	45.60±2.18	44.67±1.82	44.49±0.83

#1 = Raw fish pastes added with NaCl, MSG, and sucrose.
#2 = Raw fish pastes added with NaCl, MSG, and SHMP.
#3 = Raw fish pastes added with MSG, sucrose and SHMP.
#4 = Raw fish pastes added with NaCl, MSG, sucrose, and SHMP.
Fish balls = Cooked fish paste added with NaCl, MSG, sucrose and SHMP.
*Initial fatty acids percentage in neutral lipids of washed sucker flesh prior to added with additives.
T = Treatment, M = Month

Table 22. Means and standard deviations of TBA values** of the minced sucker products (mg malonaldehyde/kg sample).

Storage Period, months	Treatment				
	Raw				Cooked
	NaCl-MSG-Sucrose	NaCl-MSG-SHMP*	MSG-Sucrose-SHMP	NaCl-MSG-Sucrose-SHMP	NaCl-MSG-Sucrose-SHMP
0	$0.58^{a} \pm 0.28$	$0.33^{b} \pm 0.26$	$0.26^{bc} \pm 0.17$	$0.42^{ab} \pm 0.25$	$0.08^{d} \pm 0.05$
1	$1.36^{a} \pm 1.14$	$0.43^{b} \pm 0.37$	$0.35^{b} \pm 0.23$	$0.48^{b} \pm 0.34$	$0.04^{cx} \pm 0.04$
2	$1.23^{a} \pm 0.89$	$0.67^{b} \pm 0.34$	$0.49^{bx} \pm 0.26$	$0.64^{b} \pm 0.30$	$0.47^{bx} \pm 0.33$
3	$0.91^{ax} \pm 0.71$	$0.52^{b} \pm 0.36$	$0.34^{bx} \pm 0.17$	$0.48^{bx} \pm 0.29$	$0.22^{cx} \pm 0.17$
4	$1.06^{ax} \pm 0.20$	$0.66^{bx} \pm 0.25$	$0.36^{cx} \pm 0.20$	$0.65^{bx} \pm 0.24$	$0.42^{cy} \pm 0.14$
5	$1.28^{ay} \pm 1.01$	$0.67^{by} \pm 0.42$	$0.42^{cy} \pm 0.27$	$0.61^{by} \pm 0.42$	$0.44^{bcy} \pm 0.25$
6	$1.21^{ay} \pm 0.95$	0.64^{by} 0.42	$0.24^{cy} \pm 0.10$	$0.26^{cy} \pm 0.09$	$0.26^{cy} \pm 0.09$

^{abc}Figures with different superscripts have significant difference at $\alpha<0.05$ among treatments.

x Occurrence of orange interferring pigments in the TBA reacting solution.

y Occurrence of yellow interferring pigments in the TBA reacting solution.

* SHMP = sodium hexametaphosphate.

** Figures shown in this Table are the corrected TBA values calculated from the formulas which are listed in Appendix B17.

lipid oxidation while addition of NaCl may promote lipid oxidation in sucker paste system. Thus, the antioxidant effect through metal chelating action of SHMP is much greater than the prooxidant effect of NaCl, hence, the SHMP retarded the prooxidant effect of NaCl.

Occurrence of yellow and /or orange pigments was found in the TBA reaction solution of the frozen sucker paste products during frozen storage. The formation of yellow pigment could be due to complexing of aldehyde with proteins, sucrose and other food constituents that were present in sucker paste products (Turner et al., 1954; Yu and Sinnhuber, 1962). Marcuse and Johnasson (1973) reported that alkanals resulting from lipid oxidation formed a yellow pigment when reacting with TBA reagent, while only malonaldehyde, 2,4-alkadienals and, to a lesser extent, 2-alkenals produced the red 530 nm pigment.

The low concentration of TBA values found in these fish paste products could be interpreted as the result of a relatively low rate of lipid oxidation, and could be attributed to the washing of the preliminary raw sucker flesh, the presence of SHMP as a metal-chelating agent, vacuum packaging which provides an oxygen free enviroment, relatively low storage temperature (-23^{o}C), inactivation of enzymes by heat treatment for cooked fish balls, and a dark storage room. The fluctuation of TBA values from month to month could be influenced by complex factors such

as duration of the fish stored in ice prior to processing (Deng, 1978), storage condition before and after processing (Deng, 1978), mechanically deboned process (Lee et al., 1975; Lee and Toledo, 1977), kind and amount of fatty acids in the system (Shono and Toyomizu, 1971; Takama, 1974; Fisher and Deng, 1977; Toyomizu and Hanaoka, 1980a,b), presence of natural antioxidant and additives (Biggar et al., 1975; Iredale and York, 1977; Morris and Dawson, 1979), presence of matal iron and copper in muscle food system and washing water (Tappel, 1953; Castell and Bishop, 1969; Castell, 1971; Lee and Toledo, 1977; Fisher and Deng, 1977), ratio of sarcoplasmic proteins to myofibrillar proteins in the system (Shenouda, 1980; Froning, 1981), and possible incccuracies in analyses.

Changes in cholesterol.

Analyses of variance indicated a significant change in cholesterol (Table 24) in sucker paste with added MSG-Sucrose-SHMP, NaCl-MSG-Sucrose-SHMP and cooked fish balls during six months of storage at -23^{o}C. However, there were no significant differences among the 5 treatments. The slight decrease in cholesterol level shown in Table 23 could possibly be attributed to a slight oxidative reaction of cholesterol; however, this is unlikely since no evidence has been reported in the current literature to support the finding that cholesterol oxidation takes place in a food

Table 23. Changes of cholesterol level[c] in sucker paste products during 6 months of storage at -23°C (mg cholesterol/100 g sucker)

Storage time, months	Raw				Cooked
	NaCl-MSG-Sucrose	NaCl-MSG-SHMP	MSG-Sucrose-SHMP	NaCl-MSG-Sucrose-SHMP	NaCl-MSG-Sucrose-SHMP
1	$17.10^{a}\pm1.30$	$17.80^{a}\pm0.60$	$19.50^{a}\pm0.90$	$18.60^{a}\pm0.60$	$18.79^{a}\pm0.87$
2	$18.20^{a}\pm1.10$	$16.90^{a}\pm0.04$	$19.60^{a}\pm1.00$	$17.90^{a}\pm0.22$	$18.57^{a}\pm0.35$
3	$17.50^{a}\pm0.50$	$15.60^{a}\pm1.20$	$17.30^{a}\pm0.90$	$15.70^{ab}\pm0.70$	$14.79^{b}\pm0.87$
4	$16.10^{a}\pm2.00$	$14.20^{a}\pm0.80$	$14.60^{b}\pm0.90$	$13.80^{b}\pm0.30$	$14.66^{b}\pm0.44$
5	$15.50^{a}\pm0.20$	$15.20^{a}\pm1.00$	$15.80^{b}\pm0.70$	$14.70^{b}\pm1.00$	$15.70^{b}\pm1.35$
6	$16.70^{a}\pm2.30$	$15.30^{a}\pm1.30$	$16.80^{ab}\pm0.70$	$16.90^{ab}\pm3.30$	$13.92^{b}\pm0.26$

a,b Different superscript means the value under it has significant difference (at $\alpha<0.05$) during 6 months of storage.
c Average value of two determinations.

Table 24. Analyses of variance for cholesterol value and its approximate significance probability of F statistics.

Source of variance	Sum of square	Degree of Freedom	Mean Square	F Statistics	Approximate Significance Probability of F Statistics
Treatment	0.77	4	0.19	1.77	0.16
Month	6.37	5	1.28	11.79	<0.0005
Batch	0.01	1	0.01	0.09	0.77
Treatment X Month	1.49	20	0.74	0.69	0.80
Residual Error	3.14	29	0.11		

system at the freezing temperature which was used in this study. Evidence that cholesterol oxidation takes place in a food system at a frying temperature (180^{o}C) has been reported by Mai et al.(1978) using deep-fat fried fresh water fish and Ryan et al.(1981) using heated tallow for an extended period. Further study is needed to understand the possible mechanism involved in cholesterol change under different conditions. The decrease of cholesterol levels shown in Table 23 did not follow a linear relationship. The slight decline in the cholesterol concentration also may be due to random sampling and variation in the analytical procedures, since lipid oxidation in sucker paste products was not seriously reflected by low TBA values.

Sensory evaluation of flavor and texture.

The parameters of sensory scores used for this study are shown in Appendix C. Rancid and putrefactive characteristics were used to describe a specific off-flavor of the frozen fish balls. Sensory scores, shear values and TBA values are shown in Table 25 for comparison of subjective, objective, and chemical tests. Mean sensory scores for flavor acceptability decreased as the intensity of off-flavors increased with length of storage. This trend is consistent with the TBA values which increased during storage (Figure 4). Although off-flavor development occurred during storage, it should be noted that the sensory

Table 25. Sensory scores[1], TBA values, and gel strength for cooked fish balls during six months of frozen storage at -23°C.

Storage Period, Month	Gel Strength (lb. force / g sample)	Sensory Characteristics				TBA Values (mg malonaldehyde per kg sample)
		Textural Resilience	Textural Acceptability	Off-flavor[2]	Flavor Acceptability	
0	$3.30^{a}\pm0.21$	$7.74^{a}\pm0.21$	$7.17^{a}\pm0.57$	$1.00^{b}\pm0.00$	$7.64^{a}\pm0.04$	$0.08^{b}\pm0.05$
3	$2.89^{a}\pm0.16$	$6.15^{b}\pm0.52$	$6.62^{a}\pm0.29$	$2.76^{a}\pm0.10$	$7.24^{a}\pm0.24$	$0.22^{a}\pm0.17$
6	$2.50^{a}\pm0.27$	$4.95^{c}\pm0.25$	$6.38^{a}\pm0.39$	$2.47^{a}\pm0.25$	$6.97^{a}\pm0.47$	$0.26^{a}\pm0.09$

[1] Sensory scores were measured by a 9-point scale from 1 to 9 as shown in Appendix C.

[2] 5 = Detectable level on a 9-point scale.

[abc] Numbers in the same column bearing different superscript are significantly different at $\alpha<0.05$.

Table 26. Significance probability on changes in sensory characteristics of frozen fish balls during six months of storage at -23°C

Sensory Characteristics	Test of Statistics	Critical Values	$f_{0.01,\ t-1,n-t}$
Off-flavor	592.97	5.15	0.01
Textural Resilience	77.45	5.15	0.01
Textural Acceptability	2.07	5.15	None Significant
Flavor Acceptability	2.31	5.15	None Significant

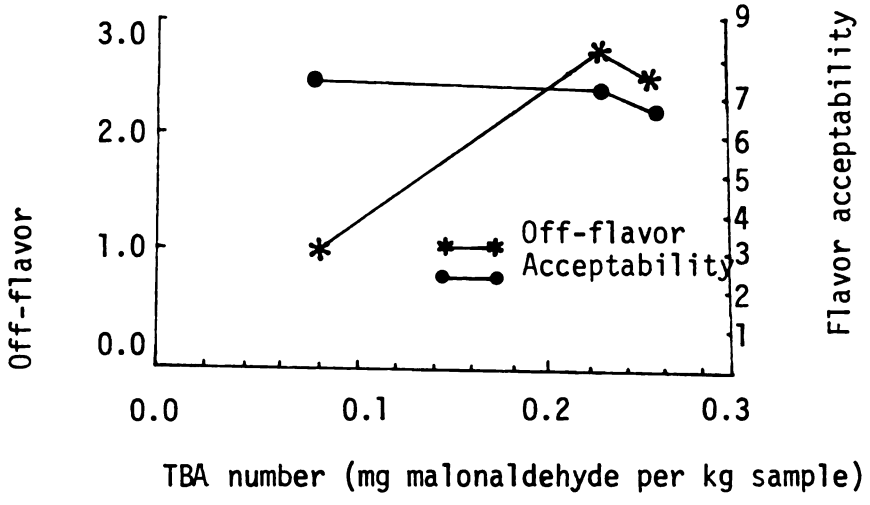


Figure 4 Correlation between off-flavor and TBA number of cooked fish balls during frozen storage at -23°C for six months.

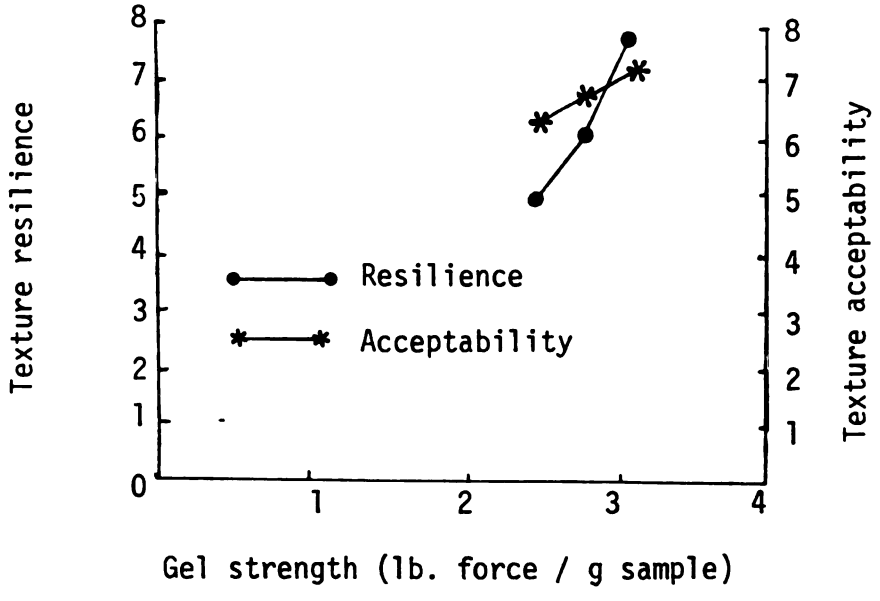


Figure 5. Correlation between gel strength and texture resilience of cooked fish balls during frozen storage at -23°C for six months.

scores for off-flavor were significantly lower than the detectable level, agreeing with the low TBA values previously reported (Table 25) and minor oxidation.

Both sensory texture scores and shear values suggest that as the length of storage increased up to 3 months, more softening occurred, making the texture of fish balls less acceptable (Figure 5). Upon further storage, no significant changes could be detected by the panelists (Table 26). After six months of frozen storage the cooked fish balls were still judged acceptable having mean sensory scores for texture and flavor of 6.38 and 6.97, respectively, based a 9 point scale.

The hydrolysis of fish muscle lipids, per se, may not have any direct influence on the taste and the quality of the minced sucker flesh, but the unsaturated fatty acids may oxidize and cause slight off-flavors while accumulated free fatty acids may cause denaturation of proteins and toughening of the texture (Anderson and Steinberg, 1964; Dyer and Dingle, 1961).

Mechanically deboned sucker flesh was formulated into paste type products containing sodium chloride (NaCl), monosodium glutamate (MSG), sucrose and sodium hexametaphosphate (SHMP). NaCl was used to extract myofibrillar proteins from sucker flesh; MSG and sucrose were used as cryoprotectants as well as flavor enhancers; SHMP was used as a swelling and binding agent as well as a metal ion scavenger

in fish paste products. Sucker paste products were vacuum packed and stored at -23^{o}C for six months. The quality of sucker paste products was evaluated in TBA values, fatty acid composition as well as sensory evaluation by taste panels.

Decrease in the relative concentration of unsaturated fatty acids was related to the changes in the polyunsaturated fatty acids in the phospholipids, rather than in neutral lipids. Hydrolysis of phospholipids was found in the PE fraction but not in the PC fraction. Although analyses of fatty acid composition in PE, PC and their lyso-derivatives were not carried out in this study, changes in the relative percentage of polyunsaturated fatty acids C21:5 and C22:6 were likely to be related to the hydrolysis of PE and decrease in relative percentage of total phospholipids in sucker paste products.

Prewashing of minced sucker flesh, preheat treatment, addition of SHMP, vacuum packaging, and a low freezing temperature significantly reduced the TBA values and prevented the development of off-flavor. However, fluctuating temperatures as low as -23^{o}C might not completely stop the enzymatic activity as the changes of polyunsaturated fatty acids occurred at -23^{o}C. Using individual quick freezing (IQF) technique may improve the product quality in the future study since IQF can inactivate enzyme activity at a much faster rate in initial freezing process. Using chilled

deionized water and /or diluted, chlorinated water (5ppm available chloride) for the washing process could be beneficial to quality stability of the raw fish flesh. Preheating of fish balls resulted in a more stable storage quality than that of raw fish pastes. Based on the results obtained from chemical tests and sensory evaluation, the quality of raw fish pastes and cooked fish balls are relatively acceptable. Other types of sausage products could be formulated from these raw fish pastes as a potential protein source.

SUMMARY AND CONCLUSIONS

In general, fish have a relatively shorter postmortem duration than the mammalian animals. The acceptable shelf life for ice-stored sucker flesh usually does not exceed one week. This varies with different species, season of harvest and size of the fish. The study on the effect of ice-storage on the myofibrillar protein extractability and gel forming ability lead to the conclusion that the myofibrillar proteins of pre-rigor sucker flesh are more extractable and provide for better gel forming ability upon cooking than those in the rigor and post-rigor stage. Myosin heavy and light chains together had a significant effect on the gel strength.

The final total yield of the washed minced sucker flesh was only 25% of the whole fish. However, the washing technique improves the product quality not only by removing the sarcoplasmic constituents, blood materials and internal organ contaminants but also by reducing a substantial amount of unbound lipids, free cholesterol content and the initial TBA values of the raw minced sucker flesh. The fatty acid ratio of total unsaturation to saturation in total lipid increased slightly through washing, while in the phospholipid fraction, a shift from more saturation to less saturation was observed. Therefore, washing the minced

sucker flesh before processing and subsequent frozen storage could be a practical way to improve the product quality and storage life. The gel forming capacity of the fish pastes could also be improved by washing raw fish muscle because washing reduced sarcoplasmic proteins and thus increased the relative concentration of myofibrillar proteins.

Mechanically deboned sucker was formulated into paste type products containing sodium chloride, monosodium glutamate, sucrose and sodium hexametaphosphate. Sodium chloride was used to extract myofibrillar proteins from sucker flesh; monosodium glutamate and sucrose were used as cryoprotectants as well as flavor enhancer; sodium hexametaphosphate was used as a swelling and binding agent as well as a metal ion scavenger in fish paste products. Sucker paste products were vacuum packed and stored at -23^{o}C for six months. The quality of sucker paste products was evaluated by changes in TBA values, fatty acid composition as well as sensory evaluation.

Decrease in the relative concentration of unsaturated fatty acids was related to the polyunsaturated fatty acids of phospholipids rather than that of neutral lipids. The changes in fatty acid content in the phospholipid fractions primarily occurred between the initial time of processing and the first month of frozen storage. Upon further storage, the changes were relatively small

suggesting that changes during frozen storage was minor. The primary changes which occurred during processing and prior to complete freezing could be caused by the prolonged exposure of raw samples to the open atmosphere at room temperature, which could promote lipid oxidation and /or lipolysis of fish pastes. Hydrolysis of phospholipids was found in the PE fraction but not in the PC fraction. Changes in the relative percentage of polyunsaturated fatty acids C21:5 and C22:6 were likely to be related to the hydrolysis of PE and decrease in relative concentration of total phospholipids in sucker paste products.

Prewashing of minced sucker flesh, pre-heating treatment, addition of SHMP, vacuum packaging, and a low freezing temperature significantly reduced the TBA values and prevented the development of off-flavor. However, fluctuating temperatures as low as -23^{o}C might not completely stop enzymatic activity as the changes of polyunsaturated fatty acids occurred at -23^{o}C, especially during the initial freezing period.

Based on the resluts obtained from chemical tests and sensory evaluation, the quality of the raw fish pastes and cooked fish balls were relatively acceptable. Because defrosting and reblending of frozen fish pastes is a necessary step before forming them into fish balls and other gel type of products, it is recommended that preheating of pastes in any form of products will result

in a more stable product quality and cost less in handling them properly than if they are stored at the raw stage. The additional blending may cause disintegration of the gel structure, resulting in a poor gel texture. In addition, lipid oxidation may occur during blending and subsequent cooking and cooling processes. A second packaging may be needed for further storage and transporting. Thus, a higher cost is needed for a two stage than that of the one stage processing.

PROPOSALS FOR FURTHER RESEARCH

The study of minced sucker paste products and their quality stability during frozen storage has raised some questions that need further investigation. These include:

1. Development of fish paste products using mixtures of more than two kinds of fish and their quality stability at frozen temperature below $-32^{o}C$.

2. Isolation of individual myofibrillar proteins from fish muscle and the effect of each individual protein on the gel forming characteristics.

3. Further investigation is needed to confirm fatty acid changes in sucker paste products caused by processing per se.

4. Whether the C16:0 acid found in phospholipids of sucker flesh is actually accumulated at Sn-2 position needs further study.

5. Further analyses of fatty acid composition in PE, PC and their lyso-derivatives as well as free fatty acid analyses will provide a better information on lipid changes in sucker paste products during frozen storage.

6. Further study is needed to provide an information for the possible mechanism which involved in the cholesterol changes under different conditions, such as temperatures, pH, and enzyme activity, etc.

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APPENDIX

Appendix A1. ELECTROPHORESIS SOLUTIONS

1. Tris-Glycine Stock Solutions (0.5 M Tris, 1.5 M Glycine)

	2 liters	1 liter	3 liters
a. 0.5 M Tris-Base (mw = 121.1)	121 grams	60.5 g x 3 =	181.5 g
b. 1.5 M Glycine (mw = 75.1)	225 grams	112.5 g x 3 =	337.5 g

Label and store at 2°C in 1-gallon plastic bottle.

2. 25% Acrylamide, 0.25% Bis (for 10% gels cross-linked with Bis)

	250 ml
a. 25% acrylamide	62.5 grams x 3 = 187.5 g
b. N,N Methylenebisacrylamide (0.25%)	0.625 grams x 3 1.875 g

Label and store at 2°C in plastic (filter through paper in Buchner funnel).

3. 25% Acrylamide, 0.6% DATD (for 10% gels, cross-linked with DATD)

	250 ml
a. 25% acrylamide	62.5 grams x 4 = 250.0 g
b. 0.5% N,N Diallytartardiamide (DATD)	1.5 grams x 4 = 6.0 g

Label and store at 2°C in plastic (filter through paper in Buchner funnel).

4. 2.5% SDS*

	500 ml
a. 2.5% sodium dodecyl sulfate	12.5 grams x 4 = 50.0 g

*DO NOT BREATE THIS STUFF!!!!

Store at room temperature

(PREPARE IMMEDIATELY BEFORE USE):

5. 1% Ammonium Persulfate

	10 ml
a. 1% ammonium persulfate	0.1 gram

6. Chamber Buffer (0.1% SDS, 0.20 M Tris-Glycine, pH 8.8)

	1 liter
a. 2.0 M Tris-Glycine	100 ml
b. 2.5% SDA	40 ml
3. H_2O	860 ml

7. Tracking Dye (dissolve sample in 50-100 µl 1.0% SDS, 0.05 M Tris-HCl, pH 7.0, 0.5% MCE, 20% glycerol, 0.01% Pyronin Y)

	100 ml
a. 1.0% SDS	40 ml of 2.5% SDA
b. 0.5 M tris Ma Base	0.61 grams of Trizma Base
c. 0.5% mercaptoethanol (hood!)	0.5 ml of 2-mercaptoethanol (under the hood!!)
d. 20% glycerol	20 ml of glycerol
e. Pyronin Y	0.01 g
f. water to 100 ml	39 ml
g. adjust pH to 7.2 with 6 N HCl	
h. store in plastic bottle in freezer	

8. Staining Solution (24 gels) (prepare immediately before use)

a.	0.1 grams Coomassie blue
b.	150 ml methanol
c.	129 ml H_2O
d.	21 ml glacial acetic acid
	300 ml

9. Destaining Solution

2 1	4 1	8 1
1,750 ml H_2O	3,500 ml H_2O	7,000 ml H_2O
150 ml glacial acetic acid	300 ml glacial acetic acid	600 ml glacial acetic acid
100 ml methanol	200 ml methanol	400 methanol

Procedure for SDS-Polyacrylamide Gel Electrophoresis (10% gels)

To pour 12, 25 or 36 - 10 cm gels:

36	24	12		Final Concentration
30	20	10.0	ml of 25% Acrylamide (Bis or DATD)	10 %
15	10	5.0	ml of Tris-glycine buffer	0.4 M
3.75	2.5	1.25	ml of Glycerol	5.0 %
3	2	1	ml of 2.5% SDS	0.1 %
.030	.020	.010	TEMED	0.04%
20.25	13.5	6.75	H_2O	----
3.0	2.0	1.0	ml of 1% ammonium persulfate (i.e., prepare 0.1 grams/10 ml H_2O) (Add this last!)	0.04%

1. Measure length of gel to be run on tube (8 cm), fill to that level, and layer with H_2O carefully.
2. Pour off H_2O after polymerization (at least 1/2 hr) and load sample.
3. Add 450 ml of chamber buffer to upper and lower chambers and run at ~0.5 mA/gel.
4. Stain; destain.

Appendix A2. Contents of the Electrophoresis calibration kits.

High Molecular Weight (HMW) Calibration Kit - The Protein Mixture in each vial consists of:

Protein	Mol. Wt.	Subunit Mol. Wt.	Ref.	Source
Thyroglobulin	669,000	330,000 18,500	a,b	hog thyroid
Ferritin	440,000	(220,000)	c,d	horse spleen
Catalase	232,000	60,000	e,f	beef liver
Lactate Dehydrogenase	140,000	36,000	g	beef heart
Albumin	67,000	67,000	h,i	bovine serum

Low Molecular Weight (LMW) Calibration Kit - The Protein Mixture in each vial consists of:

	Subunit Mol. Wt.	Ref.	Source
Phosphorylase b	94,000	j	rabbit muscle
Albumin	67,000	i	bovine serum
Ovalbumin	43,000	i	egg white
Carbonic Anhydrase	30,000	k	bovine erythrocyte
Trypsin Inhibitor	20,100	l	soybean
α-Lactalbumin	14,400	m	bovine milk

Appendix B1. Changes in proportionate percentage of fatty acid composition[a] of total lipid in sucker paste preblended with NaCl-MSG-Sucrose during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month		
	1	3	6
14:0	1.01±0.06	1.30±0.39	1.14±0.11
15:0	0.38±0.04	0.44±0.06	0.43±0.03
16:0	18.86±0.59	18.28±1.90	17.95±0.41
17:0	0.81±0.06	0.85±0.12	0.87±0.02
18:0	4.95±0.20	4.18±0.28	4.80±0.48
21:0	0.40±0.08	0.08±0.06	0.58±0.01
14:1	0.32±0.11	0.48±0.37	0.42±0.05
15:1	0.59±0.11	0.83±0.09	0.55±0.01
16:1	9.96±0.56	12.60±2.62	10.49±0.25
17:1	0.90±0.07	0.99±0.37	0.98±0.08
18:1	11.59±0.54	13.39±1.71	12.56±0.20
20:1	0.66±0.02	0.56±0.13	0.70±0.05
21:1	7.47±0.10	6.91±0.95	7.46±0.04
24:1	0.99±0.04	0.78±0.03	1.09±0.12
18:2	2.25±0.13	1.12±0.80	2.68±0.25
20:2	0.22±0.21	0.39±0.01	0.50±0.08
18:3	0.17±0.05	0.69±0.69	0.39±0.18
18:4	1.00±0.10	0.76±0.33	1.14±0.08
20:4	0.40±0.01	0.33±0.01	0.44±0.00
20:5	10.74±0.14	10.24±0.95	10.25±0.16
21:5	0.44±0.05	0.57±0.09	0.65±0.29
22:5	4.63±0.06	4.19±0.31	4.48±0.05
22:6	21.05±1.02	18.40±3.38	19.53±0.03
Total Sat.	26.41±1.03	25.13±2.81	25.77±1.06
Monoenoic	32.48±1.55	36.54±6.27	34.25±0.80
Dienoic	2.47±0.34	1.51±0.81	3.18±0.33
Polyenoic	38.43±1.43	35.18±5.76	36.88±0.79
Total Unsat.	73.38±3.32	73.23±12.84	74.31±1.92

[a]Average of two determinations from each batch.

Appendix B2. Changes in proportionate percentage of fatty acid composition[a] of total lipid in sucker paste preblended with NaCl-MSG-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month		
	1	3	6
14:0	1.14±0.23	1.20±0.21	1.03±0.06
15:0	0.40±0.05	0.39±0.02	0.42±0.05
16:0	18.54±1.12	19.07±0.37	18.89±0.93
17:0	0.80±0.08	0.75±0.05	0.86±0.04
18:0	4.75±0.48	4.48±0.05	5.34±0.46
21:0	0.45±0.12	0.33±0.02	0.43±0.02
14:1	0.41±0.21	0.29±0.06	0.37±0.02
15:1	0.70±0.03	0.50±0.31	0.85±0.07
16:1	10.95±1.62	11.20±1.58	9.85±0.04
17:1	0.87±0.31	0.86±0.08	0.99±0.05
18;1	11.96±1.12	11.99±0.91	11.96±0.13
20:1	0.73±0.10	0.57±0.09	0.65±0.06
21:1	7.40±0.51	7.39±0.26	7.24±0.22
24:1	0.91±0.03	0.87±0.11	1.05±0.00
18:2	2.32±0.19	2.08±0.11	2.38±0.21
20:2	0.40±0.01	0.37±0.04	0.41±0.01
18:3	0.16±0.08	0.10±0.03	0.15±0.01
18:4	1.03±0.17	0.95±0.01	0.96±0.02
20:4	0.35±0.01	0.34±0.05	0.36±0.03
20:5	10.76±0.62	10.98±0.18	10.37±0.44
21:5	0.39±0.04	0.40±0.04	0.42±0.02
22:5	4.51±0.23	4.54±0.18	4.66±0.04
22:6	20.12±2.23	20.63±1.21	20.42±0.74
Total Sat.	26.08±2.08	26.22±0.72	26.97±1.56
Monoenoic	33.93±3.93	33.67±3.40	32.31±0.59
Dienoic	2.72±0.20	2.45±0.15	2.79±0.22
Polyenoic	37.32±3.38	37.94±1.70	37.34±1.30
Total Unsat.	73.97±7.51	74.06±5.25	73.44±2.11

[a]Average of two determinations from each batch.

Appendix B3. Changes in proportionate percentage of fatty acid composition of total lipid in sucker paste preblended with MSG-sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month		
	1	3	6
14:0	1.30±0.37	1.17±0.27	1.01±0.02
15:0	0.44±0.08	0.40±0.03	0.36±0.02
16:0	17.59±0.27	16.54±0.17	16.72±0.12
17:0	0.93±0.17	0.84±0.08	0.83±0.04
18:0	4.95±0.01	4.98±0.17	4.43±0.20
21:0	0.81±0.40	0.59±0.19	0.56±0.01
14:1	0.55±0.20	0.50±0.15	0.39±0.03
15:1	0.64±0.02	0.75±0.01	0.39±0.19
16:1	12.17±1.96	12.01±1.99	11.58±0.33
17:1	1.14±0.27	1.06±0.14	1.03±0.06
18:1	13.03±1.43	13.30±1.70	12.70±0.39
20:1	0.92±0.17	0.92±0.14	0.82±0.03
21:1	6.60±1.10	6.89±0.91	7.22±0.30
24:1	0.90±0.12	0.96±0.17	1.00±0.02
18:2	2.69±0.29	2.71±0.31	2.78±0.27
20:2	0.37±0.03	0.49±0.09	0.39±0.01
18:3	0.29±0.15	0.24±0.09	0.13±0.10
18:4	1.27±0.29	1.19±0.23	1.16±0.06
20:4	0.35±0.07	0.39±0.05	0.84±0.33
20:5	10.06±0.97	10.24±0.82	10.86±0.13
21:5	0.69±0.09	0.59±0.17	0.77±0.30
22:5	4.12±0.50	4.49±0.37	4.44±0.08
22:6	18.12±3.13	18.80±2.92	20.01±0.38
Total Sat.	26.02±1.30	24.52±0.91	23.91±0.41
Total Unsat.	73.91±10.79	75.53±10.26	76.51±1.63
Monoenoic	35.95±5.27	36.39±5.21	35.13±1.35
Dienoic	3.06±0.32	3.20±0.40	3.17±0.28
Polyenoic	34.90±5.20	35.94±4.65	38.21±1.76

Appendix B4. Changes in proportionate percentage of fatty acid composition of total lipid in sucker paste preblended with NaCl-MSG-sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month		
	1	3	6
14:0	1.36±0.31	1.61±0.27	0.99±0.09
15:0	0.41±0.07	0.48±0.02	0.39±0.03
16:0	18:91±2.69	17.72±0.64	18.63±0.70
17:0	0.82±0.12	0.77±0.02	0.75±0.05
18:0	4.59±0.25	4.45±0.20	4.65±0.15
21:0	0,47±0.45	0.69±0.20	0.52±0.05
14:1	0.40±0.36	0.71±0.12	0.36±0.06
15:1	0.39±0.27	0.66±0.12	0.45±0.09
16:1	12.79±2.25	13.85±1.86	9.69±0.19
17:1	0.88±0.41	1.00±0.01	0.73±0.14
18:1	13.40±1.83	13.58±1.17	11.48±0.12
20:1	0.47±0.45	0.84±0.05	0.58±0.04
21:1	6.62±0.99	6.35±0.69	7.65±0.12
24:1	0.47±0.37	0.85±0.06	1.10±0.08
18:2	2.54±0.58	2.49±0.26	2.29±0.30
20:2	0.28±0.24	0.37±0.03	0.48±0.13
18:3	0.18±0.17	0.24±0.03	0.18±0.05
18:4	0.91±0.53	1.29±0.14	0.99±0.05
20:4	0,17±0.13	0.32±0.04	0.39±0.09
20:5	10.50±1.26	10.02±0.36	11.07±0.11
21:5	0.34±0.14	0.42±0.11	0.46±0.04
22:5	4.22±0.11	4.14±0.28	4.62±0.06
22:6	18.45±3,15	17.26±1.61	21.50±0.03
Total Sat.	26.56±3.89	25.72±1.35	25.93±1.07
Total Unsat.	73.01±13.24	74.38±6.94	74.02±1.70
Monoenoic	35.42±6.93	37.83±4.08	32.04±0.84
Dienoic	2.82±0.82	2.86±0.29	2.77±0.43
Polyenoic	34.77±5.49	33.69±2.57	39.21±0.43

Appendix B5. Changes in proportionate percentage of fatty acid composition of total lipid in cooked sucker paste (fish ball) preblended with NaCl-MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Carbon number	Time stored at -23°C, month					
	1	2	3	4	5	6
14:0	1.11±0.15	1.44±0.23	0.97±0.04	1.06±0.08	1.06±0.12	1.01±0.09
15:0	0.40±0.01	0.48±0.02	0.37±0.01	0.40±0.00	0.44±0.05	0.39±0.01
16:0	15.77±1.45	17.23±0.53	19.34±0.10	18.48±0.44	18.54±0.91	17.91±0.37
17:0	0.86±0.04	0.96±0.14	0.69±0.03	0.84±0.03	0.78±0.09	0.80±0.02
18:0	4.41±0.35	4.80±0.13	4.40±0.11	4.92±0.08	4.07±0.09	4.66±0.11
21:0	0.48±0.02	0.56±0.02	0.27±0.05	0.23±0.08	0.27±0.15	0.49±0.04
14:1	0.50±0.11	0.65±0.12	0.19±0.03	0.38±0.02	0.38±0.09	0.36±0.06
16:1	0.37±0.08	0.50±0.05	0.83±0.12	0.76±0.06	0.51±0.12	0.58±0.18
16:1	10.64±1.24	12.54±1.81	9.85±0.39	9.92±0.50	9.35±0.28	9.86±0.70
17:1	1.07±0.10	1.17±0.32	0.68±0.10	0.98±0.07	0.78±0.20	0.96±0.08
18:1	11.98±0.83	12.92±1.56	11.29±0.46	11.67±0.37	11.37±0.27	11.70±0.20
20:1	0.62±0.16	0.82±0.18	0.49±0.12	0.42±0.28	0.45±0.28	0.61±0.06
21:1	7.78±0.06	6.79±0.74	7.93±0.33	7.16±0.44	7.69±0.04	7.42±0.34
24:1	1.11±0.12	0.95±0.24	0.90±0.04	1.04±0.05	1.10±0.01	1.02±0.05
18:2	2.73±0.16	2.90±0.71	2.06±0.10	3.07±0.61	2.39±0.25	2.56±0.20
20:2	0.53±0.16	0.41±0.04	0.38±0.04	0.40±0.02	0.39±0.03	0.40±0.02
18:3	0.33±0.10	0.26±0.08	0.08±0.01	0.17±0.00	0.41±0.28	0.69±0.50
18:4	1.53±0.43	1.22±0.07	0.93±0.02	0.98±0.01	1.04±0.15	1.07±0.01
20:4	0.77±0.25	0.36±0.01	0.33±0.02	0.38±0.04	0.41±0.08	0.36±0.00
20:5	11.02±0.08	10.39±0.79	11.40±0.04	10.95±0.12	11.38±0.33	11.06±0.18
21:5	0.76±0.24	0.60±0.23	0.33±0.01	0.51±0.14	0.69±0.28	0.42±0.04
22:5	4.60±0.01	4.16±0.50	4.68±0.03	4.67±0.03	4.72±0.27	4.72±0.16
22:6	20.72±0.79	17.93±2.09	22.17±0.06	21.12±0.27	21.70±1.01	21.20±0.06
Total Sat.	23.02±2.02	25.47±1.07	26.04±0.34	25.93±0.71	25.16±1.41	25.26±0.64
Monoenoic	34.07±2.70	36.34±5.02	32.16±1.59	32.33±2.59	31.63±1.29	32.51±1.67
Dienoic	3.26±0.32	3.31±0.75	2.44±0.14	3.47±0.63	2.78±0.28	2.96±0.22
Polyenoic	39.73±1.90	34.92±3.77	39.92±0.19	38.78±0.61	40.35±2.40	39.52±0.95
Total Unsat.	77.06±4.92	74.57±9.54	74.52±1.92	74.58±4.54	74.76±3.97	74.99±2.84

Appendix B6. Changes in proportionate percentage of fatty acid composition of neutral lipids in sucker paste preblended with NaCl-MSG-Sucrose during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	2.77±0.08	2.59±0.01	2.75±0.01	2.81±0.06	2.71±0.28	2.63±0.04
15:0	0.53±0.02	0.55±0.03	0.48±0.10	0.44±0.10	0.43±0.13	0.52±0.02
16:0	12.03±0.30	11.69±0.10	12.43±0.09	12.83±0.02	12.78±0.11	11.99±0.19
17:0	1.06±0.01	1.07±0.05	0.85±0.32	0.57±0.01	0.73±0.03	1.01±0.01
18:0	3.32±0.08	3.26±0.06	3.32±0.11	3.29±0.03	3.39±0.03	3.32±0.04
21:0	1.24±0.18	1.57±0.15	1.13±0.01	1.11±0.02	1.25±0.18	1.18±0.08
14:1	1.36±0.01	1.40±0.06	1.42±0.08	1.21±0.16	1.41±0.05	1.37±0.05
15:1	0.59±0.04	0.62±0.03	0.54±0.08	0.50±0.60	0.47±0.05	0.52±0.01
16:1	24.46±0.05	23.48±0.24	24.74±0.13	24.78±0.23	24.51±0.18	23.37±0.12
17:1	1.48±0.51	1.96±0.01	1.87±0.16	1.66±0.03	1.59±0.11	1.83±0.06
18:1	21.24±0.22	20.92±0.92	21.08±0.15	20.89±0.09	21.03±0.26	21.59±1.16
20:1	1.84±0.11	1.94±0.06	1.74±0.13	1.73±0.19	1.72±0.12	1.54±0.44
21:1	3.47±0.25	4.18±0.34	3.34±0.06	3.39±0.05	3.37±0.06	4.12±0.62
24:1	0.49±0.09	0.42±0.01	0.57±0.28	0.09±0.05	0.84±0.56	0.50±0.01
18:2	5.08±0.17	4.26±0.05	4.58±0.30	4.71±0.12	4.76±0.18	4.48±0.38
20:2	0.61±0.24	0.71±0.02	0.33±0.03	0.21±0.11	0.41±0.05	0.57±0.16
18:3	0.49±0.08	0.63±0.06	0.49±0.05	0.43±0.01	0.46±0.06	0.44±0.02
18:4	1.92±0.14	2.15±0.08	2.00±0.05	1.85±0.07	1.99±0.12	1.92±0.01
20:4	0.36±0.08	0.39±0.02	0.24±0.03	0.20±0.10	0.30±0.01	0.85±0.54
20:5	7.09±0.07	7.39±0.25	7.64±0.31	7.72±0.31	7.40±0.46	7.20±0.13
21:5	1.28±0.06	0.77±0.09	0.28±0.01	1.16±0.83	0.84±0.49	0.45±0.04
22:5	2.48±0.04	2.50±0.05	2.47±0.13	2.76±0.08	2.61±0.08	2.64±0.07
22:6	5.33±0.12	5.48±0.16	5.93±0.16	5.73±0.37	5.78±0.08	5.69±0.30
Total Sat.	20.96±0.67	20.73±0.40	20.96±0.54	21.05±0.24	21.29±0.76	20.65±0.38
Total Unsat.	79.58±2.28	79.20±2.45	79.26±2.14	79.02±2.86	79.50±2.92	79.08±4.12
Monoenoic	54.93±1.28	54.92±1.67	55.30±1.07	54.25±0.86	54.94±1.39	54.84±2.47
Dienoic	5.69±0.41	4.97±0.07	4.91±0.33	4.92±0.23	5.17±0.23	5.05±0.54
Polyenoic	18.96±0.59	19.31±0.71	19.05±0.74	19.85±1.77	19.39±1.30	19.19±1.11

Appendix B7. Changes in proportionate percentage of fatty acid composition of neutral lipids in sucker paste preblended with NaCl-MSG-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	2.70±0.05	2.55±0.09	2.81±0.02	2.71±0.03	2.63±0.02	2.64±0.04
15:0	0.52±0.02	0.57±0.04	0.50±0.04	0.45±0.08	0.54±0.00	0.59±0.05
16:0	11.96±0.11	12.22±0.16	12.70±0.05	12.38±0.27	12.77±0.26	12.13±0.26
17:0	1.03±0.00	1.11±0.06	0.57±0.01	1.04±0.47	1.02±0.01	1.13±0.04
18:0	3.26±0.03	3.46±0.09	3.49±0.11	3.28±0.10	3.48±0.06	3.35±0.12
21:0	1.54±0.03	1.24±0.05	1.28±0.27	1.23±0.13	1.44±0.33	1.27±0.10
14:1	1.41±0.11	1.37±0.15	1.20±0.16	1.19±0.16	1.43±0.04	1.44±0.10
15:1	0.57±0.01	0.65±0.04	0.51±0.05	0.63±0.04	0.52±0.02	0.68±0.04
16:1	23.62±0.20	22.82±1.04	23.81±0.10	23.11±0.78	23.36±0.35	23.13±0.24
17:1	1.88±0.04	1.97±0.07	1.72±0.08	1.62±0.02	1.77±0.05	1.99±0.00
18:1	20.72±0.19	20.16±0.14	20.70±0.08	20.48±0.10	20.40±0.28	20.28±0.00
20:1	1.98±0.16	1.76±0.16	1.60±0.19	1.72±0.05	1.41±0.11	1.82±0.12
21:1	3.60±0.14	4.03±0.24	3.76±0.15	3.35±0.15	3.81±0.11	3.91±0.03
24:1	0.36±0.10	0.57±0.12	0.44±0.02	0.51±0.03	0.54±0.19	0.58±0.01
18:2	4.82±0.59	4.64±0.29	4.85±0.22	5.10±0.26	4.50±0.27	4.63±0.23
20:2	0.69±0.15	0.54±0.18	0.41±0.05	0.54±0.20	0.37±0.01	0.66±0.24
18:3	0.61±0.03	0.57±0.03	0.49±0.08	0.54±0.10	0.52±0.05	0.58±0.00
18:4	2.20±0.02	2.03±0.05	1.93±0.05	1.97±0.04	2.02±0.01	1.98±0.04
20:4	0.38±0.06	0.40±0.09	0.40±0.07	0.47±0.14	0.30±0.01	0.38±0.05
20:5	7.26±0.30	7.75±0.02	7.75±0.06	7.80±0.07	7.64±0.10	7.39±0.13
21:5	0.66±0.19	0.98±0.17	0.89±0.10	0.56±0.17	0.41±0.03	0.65±0.08
22:5	2.58±0.08	2.71±0.10	2.76±0.02	2.61±0.20	2.74±0.05	2.60±0.10
22:6	5.60±0.04	6.03±0.15	6.00±0.35	6.77±0.03	6.34±0.18	6.10±0.11
Total Sat.	21.01±0.24	21.35±0.50	21.35±0.50	21.09±1.08	21.88±0.68	21.11±0.61
Total Unsat.	78.94±2.34	79.22±1.83	79.22±1.83	78.97±2.54	78.06±1.86	80.79±1.52
Monoenoic	54.14±0.88	53.74±0.83	53.74±0.83	52.61±1.33	53.25±1.15	53.83±0.54
Dienoic	5.51±0.74	5.26±0.27	5.26±0.27	5.64±0.46	4.87±0.28	5.29±0.47
Polyenoic	19.29±0.72	20.22±0.73	20.22±0.73	20.72±0.75	19.97±0.43	21.67±0.51

Appendix B8. Changes in proportionate percentage of fatty acid composition of neutral lipid in sucker paste preblended with MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	2.54±0.04	2.68±0.27	2.28±0.04	2.77±0.15	2.57±0.02	2.35±0.02
15:0	0.56±0.05	0.55±0.00	0.43±0.15	0.35±0.00	0.55±0.04	0.55±0.02
16:0	12.66±0.04	12.28±0.02	13.50±0.04	14.51±0.66	13.19±0.78	12.87±0.33
17:0	1.09±0.05	1.08±0.00	0.65±0.01	0.48±0.01	0.84±0.23	1.10±0.01
18:0	3.82±0.18	3.48±0.18	3.68±0.02	3.69±0.18	3.75±0.21	3.85±0.12
21:0	1.39±0.34	1.37±0.24	1.26±0.02	0.39±0.39	1.27±0.31	1.11±0.06
14:1	1.31±0.06	1.35±0.08	0.66±0.03	0.68±0.26	1.27±0.09	1.20±0.07
15:1	0.62±0.04	0.61±0.01	0.37±0.04	0.40±0.01	0.60±0.06	0.62±0.02
16:1	22.39±0.56	23.21±1.54	21.91±0.44	22.65±0.08	21.57±0.43	20.84±0.24
17:1	2.28±0.37	1.93±0.13	1.41±0.01	1.50±0.01	1.69±0.18	1.82±0.01
18:1	20.18±0.80	20.58±0.73	19.24±0.31	20.59±0.60	20.00±0.23	19.81±0.03
20:1	1.55±0.21	1.76±0.05	1.66±0.10	1.27±0.06	1.56±0.27	1.81±0.18
21:1	4.31±0.72	3.74±0.55	4.04±0.40	5.39±1.36	4.17±0.04	4.56±0.08
24:1	0.42±0.00	1.20±0.70	1.97±1.45	1.66±1.09	1.33±0.81	0.55±0.10
18:2	4.32±0.14	4.77±0.44	4.00±0.29	3.87±0.25	4.41±0.08	4.42±0.40
20:2	0.37±0.01	0.49±0.15	0.49±0.04	0.20±0.10	0.35±0.01	0.70±0.25
18:3	0.44±0.01	0.57±0.10	0.40±0.04	0.32±0.08	0.49±0.11	0.52±0.01
18:4	1.91±0.01	2.06±0.18	1.82±0.10	1.40±0.15	1.95±0.13	1.92±0.00
20:4	0.29±0.02	0.33±0.04	0.33±0.01	0.16±0.10	0.30±0.01	0.41±0.05
20:5	7.76±0.52	7.42±0.70	7.78±0.75	7.87±0.75	7.62±0.25	7.74±0.44
21:5	0.76±0.17	0.92±0.03	2.25±1.69	0.53±0.31	0.38±0.10	0.46±0.04
22:5	2.63±0.01	2.58±0.28	2.92±0.01	2.56±0.43	2.83±0.13	3.08±0.12
22:6	6.41±0.33	5.97±0.87	6.75±0.64	6.85±1.25	6.82±0.28	7.79±0.40
Total Sat.	22.06±0.70	21.44±0.71	21.80±0.28	22.19±1.39	22.17±1.59	21.83±0.56
Total Unsat.	77.95±3.99	79.49±6.58	78.00±6.35	77.90±6.89	77.34±4.78	78.25±2.44
Monoenoic	53.06±2.76	54.38±3.79	51.26±2.78	54.14±3.47	52.19±2.11	51.21±0.73
Dienoic	4.69±0.15	5.26±0.59	4.49±0.33	4.07±0.35	4.76±0109	5.12±0.65
Polyenoic	20.20±1.08	19.85±2.20	22.25±3.24	19.69±3.07	20.39±0.99	21.92±1.06

Appendix B9. Changes in proportionate percentage of fatty acid composition of neutral lipids in sucker paste preblended with NaCl-MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	2.72±0.06	2.59±0.04	2.62±0.03	2.87±0.31	2.68±0.05	2.56±0.01
15:0	0.59±0.06	0.43±0.06	0.41±0.09	0.46±0.07	0.57±0.02	0.58±0.01
16:0	12.26±0.10	11.90±0.25	13.02±0.50	12.88±0.70	12.73±0.44	12.26±0.28
17:0	0.79±0.25	1.07±0.03	0.57±0.04	0.83±0.26	0.81±0.26	1.14±0.00
18:0	3.28±0.07	3.28±0.08	3.55±0.24	3.35±0.01	3.43±0.02	3.51±0.14
21:0	1.22±0.10	1.15±0.07	1.20±0.27	1.21±0.22	0.53±0.53	1.37±0.08
14:1	1.42±0.05	1.35±0.04	1.08±0.23	1.33±0.03	1.36±0.06	1.53±0.22
15:1	0.59±0.02	0.59±0.03	0.46±0.05	0.54±0.08	0.54±0.12	0.68±0.01
16:1	23.45±1.32	23.57±0.30	22.90±0.07	24.42±1.09	23.15±0.18	22.28±0.33
17:1	1.88±0.10	1.91±0.01	1.58±0.03	1.79±0.09	1.76±0.14	1.93±0.03
18:1	20.55±0.06	20.63±0.38	19.96±0.67	20.33±0.06	19.94±0.18	20.21±0.05
20:1	1.75±0.09	1.67±0.09	1.42±0.30	1.61±0.01	2.44±0.77	1.96±0.10
21:1	3.79±0.43	3.77±0.11	4.00±0.26	3.45±0.38	3.62±0.19	3.97±0.09
24:1	0.47±0.02	0.50±0.01	0.54±0.03	0.95±0.55	0.39±0.04	0.67±0.19
18:2	4.38±0.37	4.65±0.35	4.66±0.03	4.61±0.04	4.49±0.09	4.60±0.31
20:2	0.45±0.01	0.37±0.00	0.31±0.03	0.30±0.06	0.53±0.23	0.91±0.05
18:3	0.42±0.04	0.51±0.09	0.36±0.05	0.42±0.10	0.59±0.17	0.63±0.05
18:4	1.92±0.10	1.98±0.14	1.71±0.16	1.82±0.28	2.10±0.19	2.12±0.12
20:4	0.32±0.02	0.31±0.01	0.30±0.04	0.18±0.11	0.35±0.05	0.44±0.22
21:5	7.63±0.50	7.62±0.16	8.08±0.21	7.55±0.31	7.80±0.34	7.30±0.30
21:5	0.89±0.07	0.97±0.08	0.67±0.52	0.46±0.16	0.42±0.07	0.56±0.09
22:5	2.72±0115	2.74±0.07	3.02±0.01	2.60±0.24	2.77±0.11	2.74±0.08
22:6	6.05±0.43	6.37±0.35	7.64±0.06	6.06±0.10	7.05±0.84	6.28±0.09
Total Sat.	20.86±0.65	20.42±0.50	21.37±1.17	21.60±1.57	20.75±1.32	21.42±0.52
Total Unsat.	78.69±3.68	79.51±2.21	78.69±2.75	78.42±3.68	79.30±3.77	78.81±2.43
Monoenoic	53.90±2.09	53.99±0.97	51.94±1.64	54.42±2.28	53.20±1.68	53.23±1.02
Dienoic	4.83±0.38	5.02±0.35	4.97±0.06	4.91±0.10	5.02±0.32	5.51±0.36
Polyenoic	19.96±1.21	20.50±0.89	21.78±1.05	19.09±1.30	21.08±1.77	20.07±1.05

Appendix B10. Changes in proportionate percentage of fatty acid composition of neutral lipids in cooked sucker balls preblended with NaCl-Sucrose-MSG-SHMP during 6 months of storage at -23°C.

Fatty acid[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	2.66±0.05	2.66±0.01	2.81±0.01	2.73±0.04	2.81±0.16	2.86±0.01
15:0	0.59±0.01	0.57±0.01	0.54±0.02	0.55±0.03	0.60±0.01	0.56±0.01
16:0	12.68±0.61	12.10±0.19	12.30±0.10	11.94±0.20	11.89±0.20	12.36±0.06
17:0	1.07±0.17	1.09±0.03	0.87±0.30	0.86±0.31	1.05±0.12	1.04±0.01
18:0	3.62±0.04	3.44±0.08	3.33±0.10	3.31±0.15	3.28±0.07	3.30±0.03
21:0	1.22±0.09	1.42±0.15	1.15±0.06	1.17±0.01	1.53±0.21	1.15±0.06
14:1	1.40±0.04	1.40±0.04	1.45±0.03	1.44±0.01	1.51±0.12	1.46±0.03
15:1	0.63±0.03	0.68±0.02	0.56±0.06	0.62±0.03	0.69±0.02	0.55±0.01
16:1	23.41±0.21	23.62±0.35	24.86±0.32	24.44±0.40	24.07±0.46	24.49±0.62
17:1	1.56±0.30	2.01±0.03	1.88±0.26	1.87±0.15	1.96±0.11	1.88±0.07
18:1	20.05±0.16	20.80±0.51	21.05±0.22	21.09±0.22	20.28±0.88	21.16±0.58
20:1	1.73±0.21	1.86±0.10	1.70±0.02	1.69±0.15	1.78±0.41	1.79±0.15
21:1	3.42±0.19	3.15±0.40	3.01±0.31	3.31±0.14	3.41±0.09	3.28±0.42
24:1	0.48±0.12	0.39±0.04	0.40±0.14	0.30±0.02	0.46±0.05	0.36±0.01
18:2	4.77±0.15	4.59±0.47	4.99±0.21	5.02±0.18	5.17±0.43	4.98±0.22
20:2	0.67±0.23	0.72±0.04	0.32±0.03	0.37±0.01	0.69±0.35	0.44±0.01
18:3	0.46±0.00	0.54±0114	0.51±0.03	0.48±0.01	0.60±0.05	0.49±0.03
18:4	2.00±0.05	2.14±0.22	2.00±0.15	2.02±0.02	2.19±0.04	2.00±0.10
20:4	0.36±0.03	0.33±0.00	0.27±0.01	0.30±0.02	0.43±0.03	0.31±0.01
20:5	7.54±0.08	7.31±0.16	7.81±0.12	7.80±0.14	7.24±0.65	7.11±0.28
21:5	0.88±0.48	0.69±0.04	0.31±0.03	0.36±0.01	0.55±0.02	0.37±0.05
22:5	2.73±0.04	2.68±0.06	2.50±0.22	2.60±0.01	2.53±0.16	2.77±0.35
22:6	6.17±0.28	5.86±0.24	5.36±0.20	5.76±0.02	5.14±0.52	5.22±0.19
Total Sat.	21.84±0.87	21.28±0.47	21.00±0.59	20.56±0.74	21.16±0.77	21.27±0.18
Monoenoic	52.68±1.36	53.91±1.49	54.91±1.37	54.76±1.12	54.16±2.14	54.97±1.89
Dienoic	5.44±0.38	5.31±0.41	5.31±0.24	5.39±0.19	5.86±0.78	5.42±0.23
Polyenoic	20.14±0.96	19.55±1.05	18.76±0.76	19.30±0.23	18.68±1.47	18.27±1.01
Total Unsat.	78.26±2.70	78.77±2.95	78.98±2.37	79.45±1.54	78.80±4.39	78.66±3.13

Appendix B11. Changes in proportionate percentage of fatty acid composition of phospholipids in sucker paste preblended with NaCl-MSG-Sucrose during 6 months of storage at -23°C.

Fatty acid[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	0.45±0.01	0.54±0.06	0.44±0.02	0.55±0.06	0.63±0.08	0.65±0.12
15:0	0.24±0.02	0.37±0.04	0.25±0.44	0.33±0.07	0.35±0.03	0.43±0.03
16:0	18.77±0.40	19.71±0.30	18.78±0.59	18.91±0.30	20.30±0.67	20.12±0.42
17:0	0.72±0.03	0.80±0.02	0.72±0.01	0.80±0.05	0.70±0.11	0.80±0.10
18:0	5.18±0.16	4.60±0.01	4.75±0.23	4.46±0.18	4.29±0.75	4.59±0.33
21:0	0.13±0.03	0.17±0.05	0.09±0.02	0.38±0.09	0.33±0.75	0.14±0.04
14:1	0.00±0.00	0.09±0.05	0.00±0.00	0.12±0.05	0.09±0.04	0.15±0.05
15:1	0.65±0.05	0.50±0.20	0.61±0.00	0.66±0.00	0.63±0.03	0.64±0.01
16:1	5.41±0.04	5.64±0.14	5.22±0.04	5.52±0.09	5.95±0.34	5.46±0.06
17:1	0.62±0.03	0.66±0.05	0.60±0.04	0.72±0.05	0.53±0.19	0.57±0.22
18:1	8.58±0.31	9.42±0.10	8.40±0.06	9.15±0.15	10.00±0.12	9.87±0.49
20:1	0.43±0.02	1.51±0.08	0.36±0.01	0.39±0.25	0.23±0.12	0.60±0.08
21:1	8.93±0.19	8.41±0.20	8.41±0.12	8.38±0.13	8.73±0.59	8.49±0.05
24:1	1.78±0.24	1.57±0.07	2.32±0.27	1.88±0.05	1.38±0.04	1.24±0.00
18:2	1.62±0.21	1.89±0.26	1.52±0.09	2.03±0.14	1.71±0.15	2.00±0.21
20:2	0.55±0.09	0.61±0.14	0.44±0.04	0.76±0.13	1.05±0.52	0.73±0.15
18:3	0.03±0.03	0.10±0.02	0.00±0.00	0.09±0.09	0.08±0.03	0.10±0.04
18:4	0.61[d]±0.02	0.75[b]±0.04	0.55[e]±0.03	0.83[a]±0.05	0.64[c]±0.04	0.76[b]±0.06
20:4	0.56±0.10	0.50±0.09	0.42±0.02	0.57±0.09	0.40±0.02	0.54±0.05
20:5	12.75±0.99	11.31±0.19	11.46±0.17	11.19±0.22	11.00±0.68	11.14±0.30
21:5	1.79±0.54	1.37±0.19	3.73±0.97	2.38±0.40	0.53±0.04	0.48±0.24
22:5	5.19±0.05	5.06±0.08	5.38±0.05	5.09±0.09	5.29±0.41	4.99±0.18
22:6	25.06±0.92	25.48±0.52	25.60±0.08	24.78±0.64	25.97±0.30	25.48±0.88
Total Sat.	25.49±0.65	26.19±0.48	25.03±1.31	25.43±0.75	26.60±2.39	26.73±1.04
Total Unsat.	74.56±3.83	74.87±2.42	75.02±1.99	74.54±2.62	73.40±3.66	73.24±3.07
Monoenoic	26.40±0.88	27.80±0.89	25.92±0.54	26.82±0.77	26.94±1.47	27.02±0.96
Dienoic	2.17±0.30	2.50±0.40	1.96±0.13	2.79±0.20	2.86±0.67	2.73±0.36
Polyenoic	45.99±2.65	44.57±1.13	47.14±1.32	44.93±1.50	43.60±1.52	43.49±1.75

[abcde]Numbers of C18:4 with different superscripts are significantly different at $\alpha<0.05$.

Appendix B12. Changes in proportionate percentage of fatty acid composition of phospholipids in sucker paste preblended with NaCl-MSG-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	0.41±0.06	0.61±0.12	0.58±0.16	0.51±0.01	0.58±0.03	0.61±0.06
15:0	0.10±0.09	0.28±0.16	0.26±0.06	0.54±0.20	0.41±0.00	0.40±0.05
16:0	18.94±0.30	19.73±0.62	19.13±0.50	19.83±0.14	20.52±0.63	19.80±0.10
17:0	0.66±0.02	0.56±0.13	0.62±0.12	0.75±0.04	0.82±0.66	0.85±0.04
18:0	4.90±0.09	4.56±0.04	4.61±0.34	5.09±0.56	5.32±0.14	5.35±0.36
21:0	0.19±0.01	0.17±0.04	0.09±0.01	0.11±0.01	0.06±0.06	0.11±0.02
14:1	- ± -	0.10±0.10	0.00±0.00	0.04±0.03	0.14±0.02	0.15±0.14
15:1	0.33±0.08	0.65±0.09	0.63±0.10	0.54±0.07	0.55±0.02	0.42±0.16
16:1	5.48±0.20	5.92±1.61	4.75±0.65	5.57±0.16	5.53±0.17	5.61±0.21
17:1	0.55±0.04	0.57±0.09	0.55±0.07	0.54±0.11	0.31±0.06	0.70±0.05
18:1	8.56±0.16	9.13±0.57	9.10±0.08	9.60±0.23	9.38±0.07	9.90±0.08
20:1	0.47±0.02	0.45±0.06	0.43±0.01	0.48±0.03	0.56±0.13	0.57±0.10
21:1	9.12±0.11	8.25±0.28	8.40±0.06	8.68±0.10	8.43±0.11	8.28±0.22
24:1	2.22±0.01	1.80±0.41	1.95±0.26	1.47±0.11	1.79±0.16	1.44±0.04
18:2	1.45±0.04	1.59±0.26	1.70±0.03	1.81±0.08	1.67±0.30	1.95±0.17
20:2	0.75±0.02	0.48±0.00	0.44±0.03	0.58±0.08	0.52±0.01	0.08±0.00
18:3	0.03 ±0.03	0.03 ±0.03	0.02±0.01	0.06±0.01	0.06±0.02	0.10±0.02
18:4	0.66^{c}±0.02	0.73^{b}±0.05	0.57^{d}±0.01	0.70^{c}±0.03	0.68^{c}± 0.01	0.77^{a}±0.03
20:4	0.70±0.01	0.41±0.01	0.45±0.01	0.46±0.03	0.41±0.02	0.90±0.52
20:5	12.04±0.17	11.45±0.41	11.70±0.35	11.24±0.00	11.08±0.15	11.03±0.05
21:5	2.83±0.05	2.28±1.23	2.79±0.87	0.94±0.48	0.68±0.23	0.77±0.10
22:5	5.25±0.03	5.17±0.24	5.30±0.07	5.12±0.07	5.09±0.12	5.09±0.07
22:6	24.42±0.83	25.07±0.01	25.98±0.54	25.41±0.06	25.40±0.11	24.30±0.40
Total Sat.	25.20±0.57	25.91±1.11	25.29±1.19	26.83±0.96	27.71±1.52	27.12±0.63
Total Unsat.	74.86±1.82	74.08±5.45	74.77±3.15	73.24±1.67	72.28±1.61	72.49±2.31
Monoenoic	26.73±0.62	26.87±3.21	25.82±1.23	26.92±0.84	26.69±0.74	27.07±1.00
Dienoic	2.20±0.06	2.07±0.26	2.14±0.06	2.39±0.16	2.19±0.31	2.09±0.17
Polyenoic	45.93±1.14	45.14±1.98	46.81±1.86	43.93±0.67	43.40±0.66	43.39±1.14

[abcde]Numbers of C18:4 with different superscripts are significantly different at $\alpha<0.05$.

Appendix B13. Changes in proportionate percentage of fatty acid composition of phospholipids of sucker paste preblended with MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	0.64±0.12	0.38±0.00	0.36±0.05	0.46±0.06	0.48±0.12	0.59±0.06
15:0	0.38±0.07	0.17±0.07	0.21±0.05	0.31±0.08	0.28±0.08	0.40±0.00
16:0	19.56±0.85	19.70±0.00	19.32±0.39	19.42±1.11	20.07±0.40	20.34±0.73
17:0	2.01±1.17	0.74±0.01	0.65±0.09	0.76±0.01	0.64±0.05	0.79±0.05
18:0	4.30±1.10	5.24±0.44	5.43±0.09	4.94±0.36	5.12±0.85	5.80±0.10
21:0	0.22±0.10	0.12±0.04	0.04±0.04	0.12±0.04	0.13±0.03	0.10±0.10
14:1	0.15±0.06	- ± -	- ± -	0.03±0.02	0.06±0.01	0.11±0.06
15:1	0.62±0.02	0.32±0.05	0.45±0.18	0.57±0.02	0.32±0.26	0.49±0.09
16:1	7.29±0.53	5.43±0.48	4.86±0.23	5.65±0.15	5.53±0.59	5.24±0.06
17:1	0.79±0.07	0.52±0.00	0.39±0.20	0.62±0.02	0.35±0.01	0.46±0.16
18:1	10.75±1.09	9.65±0.50	8.35±0.24	9.06±0.66	9.40±0.39	9.30±0.06
20:1	0.50±0.10	0.39±0.01	0.38±0.01	0.43±0.05	0.40±0.02	0.41±0.13
21:1	8.06±0.27	8.47±0.11	8.58±0.01	8.42±0.01	8.55±0.16	8.61±0.07
24:1	1.20±0.02	2.00±0.16	2.15±0.29	1.87±0.16	1.34±0.05	1.34±0.12
18:2	1.73±0.50	1.57±0.05	1.55±0.32	1.76±0.11	1.45±0.01	1.45±0.43
20:2	0.54±0.13	0.44±0.02	0.45±0.09	0.49±0.08	0.46±0.01	0.59±0.10
18:3	0.11±0.06	0.04±0.03	- ± -	0.06±0.01	0.35±0.30	0.07±0.05
18:4	0.75[a]±0.08	0.59[c]±0.11	0.56[d]±0.03	0.65[b]±0.01	0.63[b]±0.01	0.68[b]±0.10
20:4	0.46±0.06	0.40±0.01	0.38±0.05	0.44±0.03	0.37±0.01	0.39±0.06
20:5	11.25±0.83	11.14±0.04	11.24±0.06	11.10±0.11	10.99±0.14	11.06±0.22
21:5	0.58±0.09	2.50±0.04	3.72±0.20	2.26±0.39	2.43±0.13	0.53±0.13
22:5	4.64±0.09	5.18±0.06	5.45±0.37	5.15±0.09	5.13±0.09	5.10±0.04
22:6	23.50±0.40	25.80±0.12	25.97±0.33	25.46±0.25	26.41±0.85	26.18±0.38
Total Sat.	27.11±3.41	26.35±0.56	26.01±0.71	26.01±1.66	26.72±1.53	28.02±1.04
Total Unsat.	72.92±4.40	74.44±1.79	74.48±2.62	74.03±2.13	74.17±3.03	72.01±2.25
Monoenoic	29.36±2.16	26.89±1.31	25.16±1.17	26.66±1.05	25.95±1.49	25.96±0.74
Dienoic	2.27±0.63	2.01±0.07	2.00±0.41	2.25±0.19	1.91±0.01	2.04±0.53
Polyenoic	41.29±1.61	45.65±0.41	47.32±1.04	45.12±0.89	46.31±1.53	44.01±0.98

[abcd] Numbers of C18:4 with different superscripts are significantly different at $\alpha<0.05$.

Appendix B14. Changes in proportionate percentage of fatty acid composition of phospholipids in sucker paste preblended with NaCl-MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	0.55±0.03	0.45±0.02	0.44±0.01	0.45±0.03	0.35±0.09	0.55±0.01
15:0	0.36±0.01	0.32±0.01	0.29±0.04	0.20±0.01	0.29±0.05	0.42±0.01
16:0	19.13±0.83	20.01±0.18	19.51±0.21	20.75±0.11	21.51±1.07	20.85±0.45
17:0	0.81±0.01	0.72±0.03	0.78±0.03	0.66±0.02	0.71±0.01	0.77±0.05
18:0	5.31±0.29	5.15±0.39	5.70±0.21	4.31±0.06	4.91±0.38	5.10±0.60
21:0	0.34±0.10	0.22±0.09	0.11±0.02	0.09±0.01	0.10±0.02	0.24±0.12
14:1	0.12±0.01	0.06±0.05	0.03±0.02	- ± -	0.02±0.01	0.10±0.03
15:1	0.61±0.12	0.37±0.27	0.50±0.13	0.23±0.02	0.33±0.17	0.42±0.13
16:1	5.66±0.28	5.08±0.33	5.11±0.04	5.37±0.18	5.66±0.02	5.66±0.16
17:1	0.70±0.01	0.64±0.01	0.61±0.01	0.43±0.14	0.38±0.02	0.45±0.13
18:1	9.47±0.07	9.22±0.09	8.66±0.08	8.66±0.06	9.12±0.30	9.71±0.28
20:1	0.65±0.01	0.59±0.02	0.44±0.08	0.37±0.00	0.43±0.00	0.44±0.09
21:1	8.25±0.14	8.39±0.04	8.62±0.03	8.49±0.27	8.66±0.08	8.57±0.05
24:1	1.47±0.16	2.17±0.69	1.76±0.36	2.86±0.14	1.60±0.25	1.27±0.05
18:2	1.96±0.13	1.76±0.09	1.59±0.14	1.40±0.02	1.63±0.04	1.59±0.33
20:2	0.73±0.14	0.68±0.17	0.48±0.06	0.44±0.02	0.41±0.06	0.54±0.06
18:3	0.17±0.05	0.16±0.08	0.05±0.03	0.04±0.04	0.03±0.03	0.06±0.02
18:4	0.91^{a}±0.10	0.80^{b}±0.02	0.59^{d}±0.05	0.60^{d}±0.04	0.62^{cd}±0.04	0.67^{c}±0.04
20:4	0.51±0.13	0.59±0.15	0.42±0.03	0.40±0.00	0.38±0.05	0.40±0.06
20:5	11.69±0.45	11.23±0.10	11.24±0.03	11.16±0.26	11.51±0.03	11.22±0.03
21:5	1.16±0.53	1.85±0.04	2.07±1.00	2.26±0.09	1.08±0.52	0.40±0.04
22:5	4.95±0.06	4.92±0.11	5.10±0.10	5.56±0.09	5.03±0.20	5.08±0.08
22:6	24.54±0.07	25.16±0.83	25.99±0.93	25.29±0.57	25.23±1.17	25.62±0.31
Total Sat.	26.50±1.27	26.87±0.72	26.83±0.52	26.46±0.24	27.87±1.62	27.93±1.24
Total Unsat.	73.55±2.46	73.67±3.09	73.26±3.12	73.56±1.94	72.12±2.99	72.20±1.89
Monoenoic	26.93±0.80	26.52±1.50	25.73±0.75	26.41±0.81	26.20±0.85	26.62±0.92
Dienoic	2.69±0.27	2.44±0.26	2.07±0.20	1.84±0.04	2.04±0.10	2.13±0.39
Polyenoic	43.93±1.39	44.71±1.33	45.46±2.17	45.31±1.09	43.88±2.04	43.45±0.58

[abcd] Number of C18:4 with different superscripts are significantly different at $\alpha<0.05$.

Appendix B15. Changes in proportionate percentage of fatty acid composition of phospholipids in cooked sucker paste (fish balls) preblended with NaCl-MSG-Sucrose-SHMP during 6 months of storage at -23°C.

Fatty acids[1] carbon number	Time of storage at -23°C, month					
	1	2	3	4	5	6
14:0	0.43±0.10	0.43±0.01	0.41±0.03	0.47±0.07	0.47±0.02	0.46±0.03
15:0	0.28±0.07	0.25±0.01	0.22±0.01	0.28±0.10	0.32±0.03	0.34±0.01
16:0	17.60±1.93	19.02±0.35	18.14±0.28	19.76±0.14	20.19±0.01	20.22±0.03
17:0	0.69±0.01	0.70±0.02	0.69±0.01	0.64±0.17	0.76±0.02	0.72±0.02
18:0	6.34±0.88	5.39±0.14	5.13±0.53	4.90±0.01	4.84±0.02	4.39±0.01
21:0	0.21±0.05	0.11±0.01	0.10±0.01	0.21±0.20	0.08±0.03	0.12±0.01
14:1	0.07±0.04	0.01±0.00	0.04±0.01	0.06±0.04	0.03±0.03	0.03±0.03
15:1	0.75±0.01	0.60±0.05	0.44±0.22	0.39±0.10	0.40±0.11	0.38±0.10
16:1	4.98±0.57	5.08±0.15	5.31±0.04	4.87±0.44	5.50±0.15	5.70±0.20
17:1	0.58±0.04	0.58±0.03	0.46±0.07	0.42±0.02	0.55±0.03	0.49±0.03
18:1	9.02±0.12	8.66±0.11	9.23±0.04	9.22±0.17	9.44±0.35	10.02±0.26
20:1	0.57±0.07	0.41±0.02	0.44±0.04	0.52±0.11	0.38±0.01	0.49±0.05
21:1	8.72±0.12	8.23±0.13	8.67±0.27	8.97±0.36	8.65±0.05	8.33±0.18
24:1	1.33±0.08	1.65±0.10	1.69±0.20	1.74±0.22	1.73±0.09	1.45±0.19
18:2	2.28±0.47	1.70±0.10	1.74±0.11	1.65±0.30	1.68±0.02	1.67±0.05
20:2	0.82±0.03	0.48±0.03	0.65±0.08	0.52±0.07	0.46±0.01	0.53±0.03
18:3	0.98±0.93	0.06±0.01	0.03±0.02	0.04±0.04	0.02±0.02	0.06±0.01
18:4	0.84[a]±0.16	0.66[c]±0.04	0.66[c]±0.02	0.39[e]±0.31	0.61[d]±0.03	0.73[b]±0.01
20:4	0.51±0.07	0.43±0.02	0.45±0.02	0.44±0.04	0.43±0.01	0.43±0.01
20:5	11.08±0.47	12.01±0.29	11.68±0.39	11.89±0.53	11.71±0.04	11.58±0.04
21:5	0.60±0.01	2.68±0.07	1.49±0.46	1.82±0.58	1.24±0.62	1.06±0.43
22:5	5.20±0.26	5.13±0.02	5.39±0.04	5.19±0.19	5.19±0.04	5.20±0.05
22:6	26.24±0.86	25.76±0.14	26.87±0.90	25.83±0.09	25.47±1.06	25.43±0.55
Total Sat.	25.55±3.04	25.90±0.54	24.69±0.87	26.26±0.69	26.66±0.13	26.25±0.11
Monoenoic	26.02±1.05	25.22±0.49	26.74±0.89	26.19±1.66	26.68±0.82	26.89±1.04
Dienoic	3.10±0.50	2.18±0.13	2.39±0.19	2.17±0.37	2.14±0.03	2.20±0.08
Polyenoic	45.45±2.76	46.73±0.59	46.57±1.85	45.60±2.18	44.67±1.82	44.49±0.83
Total Unsat.	74.57±4.31	74.13±1.21	75.70±2.93	73.96±4.21	73.49±2.67	73.58±1.95

[abcde] Number of C18:4 with different superscripts are significantly different at $\alpha<0.05$.

Appendix B16. Approximate significance probability of F statistics on changes in fatty acid composition with various treatment during 6 months of storage at -23°C.

Fatty Acids	Source of Variation[a]											
	Total Lipids				Neutral Lipids				Phospholipids			
	T	M	B	TM	T	M	B	TM	T	M	B	TM
14:0	0.36	0.20	0.01	0.69	0.01	0.12	0.02	0.22	0.03	0.09	0.22	0.18
15:0	0.44	0.95	0.07	0.70	0.21	0.02	0.94	0.79	0.55	0.01	0.21	0.04
16:0	0.31	0.93	0.62	0.56	0.01	0.01	0.23	0.07	0.06	0.01	0.35	0.99
17:0	0.48	0.35	0.06	0.77	0.49	0.01	0.38	0.85	0.40	0.35	0.52	0.30
18:0	0.16	0.08	0.01	0.05	0.01	0.61	0.47	0.47	0.26	0.58	0.29	0.23
21:0	0.16	0.36	0.06	0.54	0.23	0.16	0.14	0.14	0.18	0.12	0.65	0.48
14:1	0.67	0.80	0.04	0.59	0.01	0.02	0.18	0.50	0.36	0.01	0.11	0.04
15:1	0.58	0.19	0.83	0.32	0.01	0.01	0.13	0.04	0.06	0.33	0.45	0.40
16:1	0.26	0.16	0.02	0.68	0.01	0.12	0.59	0.42	0.50	0.16	0.52	0.36
17:1	0.68	0.89	0.12	0.83	0.67	0.07	0.46	0.21	0.42	0.03	0.17	0.44
18:1	0.32	0.35	0.03	0.71	0.01	0.89	0.81	0.91	0.83	0.01	0.72	0.02
20:1	0.15	0.99	0.21	0.72	0.61	0.59	0.59	0.60	0.28	0.10	0.10	0.39
21:1	0.16	0.53	0.01	0.59	0.01	0.63	0.50	0.62	0.55	0.13	0.41	0.01
24:1	0.43	0.09	0.85	0.28	0.01	0.79	0.05	0.88	0.38	0.01	0.34	0.05
18:2	0.07	0.03	0.59	0.23	0.01	0.99	0.01	0.66	0.21	0.52	0.01	0.32
20:2	0.91	0.78	0.56	0.70	0.69	0.01	0.90	0.44	0.04	0.15	0.85	0.14
18:3	0.36	0.80	0.70	0.40	0.18	0.03	0.03	0.55	0.65	0.37	0.25	0.47
18:4	0.58	0.68	0.20	0.60	0.04	0.01	0.09	0.47	0.66	0.02	0.42	0.17
20:4	0.07	0.34	0.07	0.06	0.58	0.11	0.60	0.54	0.15	0.16	0.14	0.60
20:5	0.25	0.89	0.01	0.54	0.51	0.26	0.02	1.00	0.17	0.20	0.86	0.47
21:5	0.20	0.65	0.90	0.67	0.58	0.36	0.40	0.50	0.81	0.01	0.26	0.08
22:5	0.26	0.33	0.20	0.70	0.15	0.58	0.19	0.68	0.61	0.03	0.02	0.19
22:6	0.24	0.51	0.01	0.61	0.01	0.47	0.33	0.17	0.25	0.05	0.14	0.68
% Sat. Fatty Acids	0.17	0.98	0.16	0.28	0.01	0.36	0.84	0.45	0.01	0.01	0.11	0.90
% Unsat. Fatty Acids	0.15	0.99	0.14	0.34	0.01	0.49	0.86	0.63	0.01	0.01	0.13	0.85
% Mono. Acids	0.43	0.37	0.03	0.78	0.01	0.79	0.54	0.45	0.59	0.04	0.99	0.10
% Dien. Acids	0.13	0.04	0.75	0.25	0.02	0.71	0.02	0.85	0.04	0.17	0.03	0.27
% Poly. Acids	0.09	0.36	0.01	0.55	0.02	0.84	0.09	0.68	0.28	0.01	0.69	0.19

[a] T = Treatment, M = Month, B = Batch, TM = Treatment X Month.

Appendix B17. The formula for computing corrected TBA values of sucker products.

Code #	% Transmittance			Correct extinction at 532 nm (or corrected TBA value)[a]
	$Y_{532\ nm}$	$Y_{460\ nm}$	$R=Y_{532}/Y_{460}$	
11/18 Fish Balls	83^{1}	79^{0}	0.7773	$\frac{K_{532} - 0.7773\ k_{460}}{0.9145}$
11/18 #1[b]	80^{0}	81^{2}	0.6651	$\frac{K_{532} - K_{460}.0.06651}{0.9268}$
11/18 #2[c]	85^{0}	76^{3}	0.6144	$\frac{K_{532} - 0.6144K_{460}}{0.9324}$
11/18 #3[d]	83^{0}	74^{2}	0.6330	$\frac{K_{532} - 0.6330K_{460}}{0.9304}$
11/18 #4[e]	82^{1}	69^{3}	0.5419	$\frac{K_{532} - 0.5419K_{460}}{0.9404}$
11/22 Fish Balls	87^{0}	79^{0}	0.5330	$\frac{K_{532} - 0.5330K_{460}}{0.9414}$
11/22 #1[b]	66^{0}	60^{3}	0.8341	$\frac{K_{532} - 0.8341K_{460}}{0.9082}$
11/22 #2[c]	62^{0}	$55^{1}2$	0.8056	$\frac{K_{532} - 0.8056K_{460}}{0.9114}$
11/22 #3[d]	82^{3}	73^{3}	0.6218	$\frac{K_{532} - 0.6218K_{460}}{0.9316}$
11/22 #4[e]	74^{1}	68^{0}	0.7719	$\frac{K_{532} - 0.7719K_{460}}{0.9151}$

[a]Calculation for the corrected extinction formula:

e.g. 11/18 Balls $x = K_{532} - 0.7773\ y$ --- (1)

$y = K_{460} - 0.11\ x$ ----- (2)

combine formula (1) & (2), then

$x = \frac{K_{532} - 0.7773K_{460}}{0.9145}$

[b]Raw fish paste added with NaCl-MSG-Sucrose.

[c]Raw fish paste added with NaCl-MSG-SHMP.

[d]Raw fish paste added with MSG-Sucrose-SHMP.

[e]Raw fish paste added with NaCl-MSG-Sucrose-SHMP.

Appendix C. SENSORY EVALUATION FORM

Name:_____ Date:_____

You are given 2 samples for this sensory evaluation. Please mark down the sample number on the horizontal line for the most appropriate strength of each quality characteristic.

1. Rancid odor:
   Not detectable — Detectable — Strong

2. Putrifactive odor:
   Not detectable — detectable — strong

3. Putrifactive flavor:
   Not detectable — detectable — strong

4. Rancid flavor:
   Not detectable — detectable — strong

5. Texture resiliance:
   Weak — strong

6. Acceptability:
   Texture: Not acceptable — acceptable

   Flavor: Not acceptable — acceptable