SELECTED TREATMENTS AND PROPERTIES OF MECHANICALLY DEBONED CARP (*Cyprinus carpio*) FLESH

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ABSTRACT

SELECTED TREATMENTS AND PROPERTIES OF
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Lake Huron carp were mechanically deboned and subjected to 3 topics of study: (1) efficiency of deboning operation and characteristics of minced flesh, (2) effect of 2 antioxidants on lipid stability during frozen storage and, (3) effect of 6 treatments on color, flavor and other characteristics of the flesh. Deboning yielded 42% mince containing 13-25% fat. Tenox 2 was more effective than Freez-gard as an antioxidant. Washing minced flesh with water reduced yield, increased shear resistance, reduced color intensity, heme pigment content and flavor intensity, and appeared to lower TBA numbers during storage. Addition of $NaHCO_3$ to the wash water was more effective than plain water or a NaCl wash at decreasing red color and storage TBA numbers. The NaCl wash or the addition of 5% vegetable fat to water washed mince improved overall acceptability. Hexane extraction or H_2O_2 bleaching was not successful.

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CHAPTER I

INTRODUCTION

During the past 100 years the commercial fishing industry in the Great Lakes has been characterized by shifts in the fish species targeted and has experienced significant fluctuations in landed tonnage. Stocks have been affected by the extent of exploitation, alteration of habitat, the introduction of parasitic and competitive species, the development of modern fishing methods and probably the release of toxic compounds. For most of its history the Michigan industry was supported primarily by catches of lake herring, lake trout and lake whitefish. Total production reached a peak of 47.5 million pounds per year during 1905-1909 and has generally declined to the current level of approximately 12 million pounds per year, now largely composed of alewife and lake whitefish (Tainter and White, 1977).

The economic condition of the Great Lakes commercial fishery would be strengthened by expanding the market and value of underharvested species, including sucker, carp, buffalo, burbot, sheepshead, smelt and menominee. Carp harvested in 1977 totaled just 4,800,000 pounds nationwide and 1,141,000 pounds from Michigan waters. However, these figures could probably be increased by expanding fishing effort in shallow water areas of Lake Michigan, Lake Huron and lower Great Lakes (Mattingly and Kevern, 1979).

Carp is considered a valuable food fish in eastern Europe, Asia and Japan but demand is generally low in U.S. markets. The advent of mechanical deboning equipment and recent advances in seafood technology should help to overcome problems with fine bones, earthy flavors or storage stability. Since very little information is available on the suitability of carp for use in the deboned, minced form, a preliminary research project was developed to characterize minced Lake Huron carp flesh and to control anticipated deficiencies as a basis for subsequent product development studies.

The investigation described in the following report consists of three study topics: (1) the efficiency of meat-bone separation, and the composition and quality of freshly minced carp; (2) the effect of frozen storage on lipid oxidation with and without commercial antioxidants; and (3) the effectiveness of six treatments designed to lighten color, reduce flavor and/or extend frozen storage life.

CHAPTER II

REVIEW OF LITERATURE

Underutilized Fish and Rationale for Mechanical Deboning

In recent years the United States has been faced with an increased demand for fishery products accompanied by a general decline in the availability of traditional, high value species of finfish and shellfish. In addition to an increase in population, per capita consumption of fish rose from approximately 11 pounds as recently as 1970 (Miyauchi and Steinberg, 1971) to 13.7 pounds in 1979 (USDA 1980). For economic reasons much of the potential U.S. catch is not harvested (Steinberg, 1974) and most of the production of herring-like fishes is processed to make animal feed and industrial products rather than used for human food (Lee, 1963; Okada et al., 1973). Baker (1978) estimated that 70 percent of marine fishes and 80 percent of Great Lakes species are underused. He also noted that a substantial portion of those fish captured incidental to target species are discarded due to the absence of established markets. Even for traditionally harvested species the amount of edible flesh which is recovered may be only 50 percent of what is technologically attainable (Steinberg, 1974; Nobel, 1973).

Many authors have proposed the adoption of deboning-mincing equipment and related technology to more completely utilize fishery resources (Keay, 1979; King and Carver, 1971; Lanier and Thomas,

1978). A method for mechanical deboning of fish was developed in Japan circa 1940 and has been an important process there since the early 1950s (Anon. 1970; King and Carver, 1971).

Fish are usually headed, gutted and split then passed through a machine which forces muscle particles through a perforated drum, either by squeezing the fish between the drum and a pressure belt or by constricting the fish between the drum and an auger that applies increasing pressure as it turns. This results in separation of minced nearly boneless flesh on one side of the drum from bones, scales and skin on the other side. The design and operation of these machines is summarized by Lanier and Thomas (1978).

Certain species of fish may be underutilized due to the presence of intermuscular bones which are difficult to remove by hand or by mechanical filleting (Dawson et al., 1978; Noble, 1974). Odd shaped species, such as those with unusually large heads or spines, are also difficult to process and are characterized by low fillet yields (Bremner, 1977). Keay (1979) and Teeny and Miyauchi (1972) indicated that small species of fish may not be economically processed by conventional methods. Unpleasant species appearance, name, flesh color, flavor or social connotation are other frequently suggested explanations for poor demand and underharvest of certain species (Baker, 1978; King, ca 1978; Teeny and Miyauchi, 1972; Patashnik, 1974).

Mechanical deboning is generally effective at bone removal even from notably boney species. Zapata (1978) recovered minced sucker containing 0.13 percent bone, and Apolinario (1975) determined

0.16 percent bone in mechanically separated tilapia flesh. These levels of bone content are less than what might be expected in fillets (Baker, 1978). The minced form allows for incorporation of various ingredients and additives, and permits extrusion or molding into assorted shapes. Such flexibility may serve to reduce problems of acceptability associated with species identity or stability (Bligh and Regier, 1976), flavor, texture (Hewson and Kemmish, 1976) or color (Jaurequi, 1978; Moledina et al., 1977).

Yield

Yield of mechanically deboned flesh depends on fish species and form, and machine operation. Yields ranging from 33 to 58 percent of round weight are commonly reported for various species (Reseck and Waters, 1979; Kudo, et al., 1973). Okada et al. (1973) reported a low of 28 percent yield for cod and a high of 66 percent for herring. According to Miyauchi and Steinberg (1971) the yield of minced cod may be partially affected by the high percentage of bone and skin in that species (16.7% compared to a normal of approximately 11%). However, Crawford et al. (1972_b) achieved a yield of 40.4 percent of round weight as minced cod. The reason for this discrepancy in reported yield is unclear. A small, boney African species, bongo fish, may yield 70 percent minced flesh (Noble, 1974).

Removal of bones and other waste by hand filleting results in approximately 25-30 percent fillet yield for most species (Miyauchi and Steinberg, 1971). Crawford et al. (1972_b) reported an increased yield of 38.5 percent for mechanically deboned English sole compared to fillet weight and a 79.6 percent increase for Dover sole.

Filleting wastes have been processed in a mechanical deboner to yield 31.2 percent flesh from ocean perch frames and 72.2 percent flesh from small pollock frames (King and Carver, 1971). Moledina et al. (1977) estimated 60 percent as a typical yield from frames. Lobster bodies, which are traditionally discarded during processing, yield up to 55 percent edible tissue when passed through a mechanical deboner (Noble, 1973).

Adjusting machine belt tension or drum perforation size affects the efficiency of meat-bone separation. Yield of minced flesh from headed and gutted black rockfish varied from 33 percent under "light" belt pressure to 47 percent under "medium belt pressure (Miyauchi et al., 1975). Bremner (1977) found that drum perforation sizes of 5mm and 10mm resulted in respective yields of 55.6 percent and 60 percent from a headed and gutted Australian fish species.

Composition

Average proximate composition of the edible portion of some fish species are listed in Table 1. For a given species composition may vary due to nutritional status (Cutting, 1969), season (Finne et al., 1980) or location (Aitken, 1976). Certain species taken from deep water may have abnormally high moisture:protein ratios during extended spawning periods (Aitken, 1976; U.S.D.C., 1980). Sablefish caught at 320 fathoms contained 71.0 percent water and 11.5 percent protein while those caught at 450 fathoms contained 81.8 percent water and 8.7 percent protein (U.S.D.C., 1980). Such fish are soft in texture and are sometimes known as "slinks" (Waterman, 1980).

TABLE 1.--Proximate composition of fish.

Species	Moisture,%	Protein,%	Fat,%	Ash,%	Author
(many)	70-80	15-20	0.5-20	--	Merritt, (1974)
sucker	80.7	16.6	2.0	--	Dawood, (1979)
cod	81.8	16.9	0.2	1.0	Bello and Pigott, (1979)
herring	72.3	15.6	11.5	1.3	Bello and Pigott, (1979)
salmon	67.0	17.0	14.0	--	Cutting, (1969)

The composition of normally fatty marine fish, such as herring and mackerel, may change dramatically with season. Aitken (1976) noted that herring sometimes range from less than 1 percent fat and 80 percent water to 30 percent fat and 54 percent water. Composition of mechanically deboned freshwater sucker, a lean species, varied but was not significantly related to season or location (Dawson et al., 1978). Fat content of fish is generally highest in tissue from belly, lateral line and back midline regions (Duttweiler, 1978, Rippen, 1980).

The process of mechanical flesh separation may alter composition. Crawford et al. (1972_a) reported significantly lower moisture contents after deboning in four of six examined marine species when compared to intact fillets. For five of the six species fat content was significantly higher after deboning. The authors attribute the difference to machine inclusion of subcutaneous fat, which is normally removed during hand filleting, and to moisture lost during machine separation. Similar results were reported by Webb et al. (1976).

Wong et al. (1978) recovered minced fish through four drum orifice sizes and recorded a small inverse relationship to moisture

content. The authors suggest that small (2mm) perforation diameter has no appreciable effect on passage of water but inhibits passage of solids.

Color

Color of fish flesh is primarily related to the content of the heme pigments, myoglobin and hemoglobin, and in some species to carotenoid content. Less important intrinsic pigments in fish flesh include cobalamine (a cobalt-containing porphyrin), flavins (yellow coenzymes) and cytochromes (a class of heme pigments). The relative content of heme pigments reported in mullet fillets was 63 percent myoglobin and 37 percent hemoglobin (Silberstein and Lillard, 1978) compared to approximately 90 percent of the pigment attributed to myglobin in well bled red meat (Lawrie 1966, Price 1981).

Redness caused by accumulation of carotenes is considered desirable in products made from salmon (Schmidt and Cuthbert, 1971) and shrimp (Simon, 1971). However, King (1974, ca 1978) noted that heme color is undesirable in fish sticks and portions which are traditionally produced from white fish. Federal standards for minced fish blocks downgrade color in these products (Fed. Register, 1979). Searobin fish is considered too dark for use in Newburg or chowder (Baker and Darfler, 1979).

Combining diagrams by Giffee et al. (1960) and Rust and Olson (1973) results in the scheme for common color states of myoglobin in red meat shown in Figure 1. Dehydration and pH affect color intensity, and chemical form varies with oxygen partial pressure, presence of

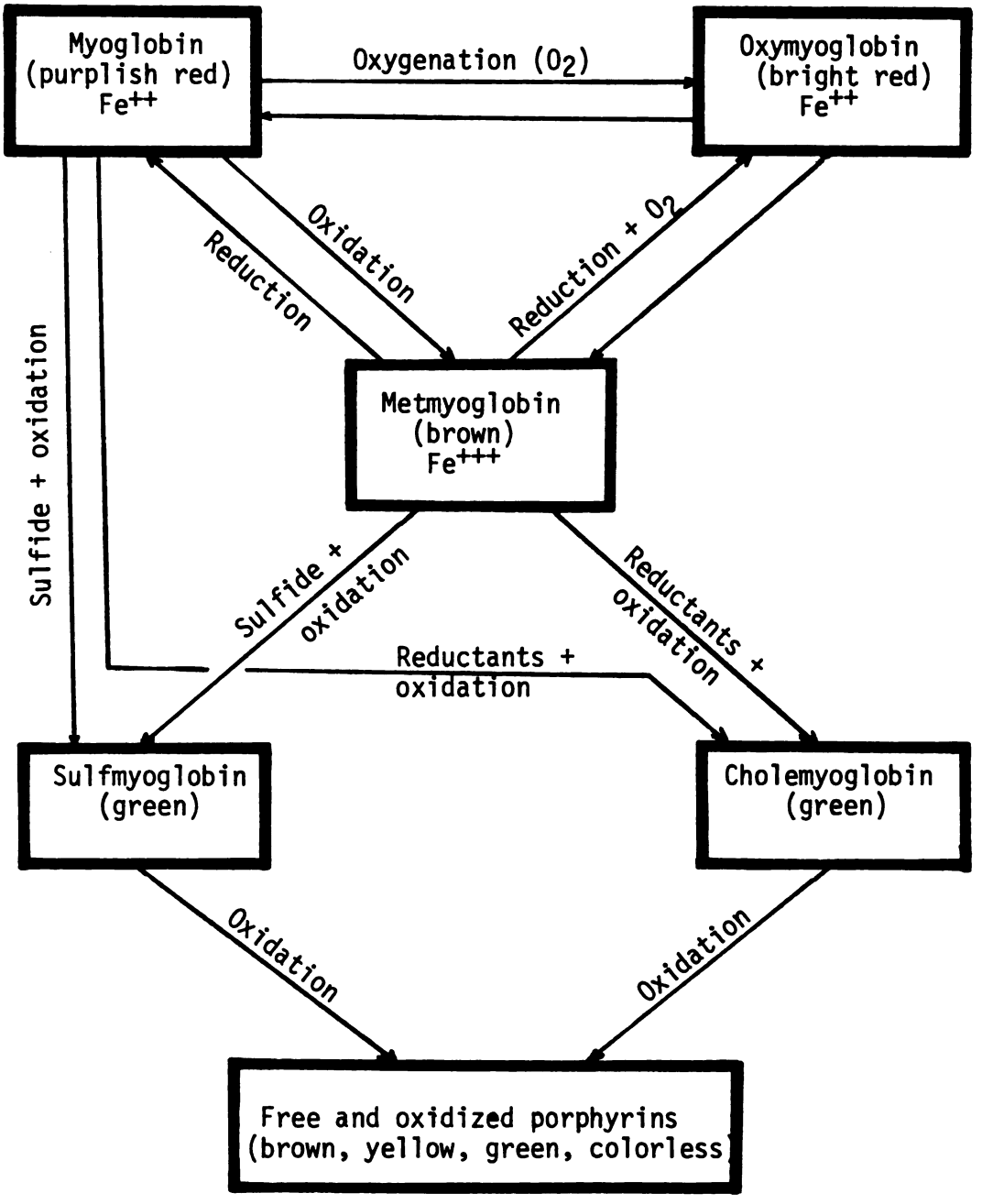

Figure 1. Chemical forms of myoglobin as they affect color of meat.

reducing agents, oxidizing agents or heavy metals, bacterial contamination and temperature (Giffee et al., 1960; Rust and Olson, 1973).

Heme content variability among fish is usually related to extent of physical exertion. Migratory or pelagic species possess a greater proportion of dark muscle and higher concentrations of myoglobin and hemoglobin than do more sedentary species (Love, 1975; Mai and Kinsella, 1979; Simidu, 1961). A similar relationship exists for red meat animals (e.g. Rickansrud, 1967).

Color problems may be significant in mechanically deboned fish, in part due to a major artery and vein system closely associated with the backbone (Lagler et al., 1962). Fish frames are known to release blood when pressed, especially from under the backbone (King, 1974). This observation may explain the findings of Silberstein and Lillard (1978) who reported an increase in hemoglobin content following deboning. Lee and Toledo (1977) suggested that contact with iron surfaces on deboning machines may catalyze oxidation of myoglobin to the brown metmyoglobin form.

Discoloration may also result from reaction of metals with amine, sulfhydryl and phenolic groups (Moledina et al., 1977). Metal and light catalyzed browning was observed in minced whiting by Anderson and Mendelsohn (1971). Maillard (nonenzymatic) browning is known to occur in fish by reaction of amino acids with either glucose, sugars arising from nucleotide degradation or carbonyls produced during lipid oxidation (Markakis, 1979; Pedraja, 1970; Ramamurthy et al., 1976).

Mechanical deboning occasionally incorporates pieces of dark skin or peritoneum in the mince (Lanier and Thomas, 1978). Jauregui and Baker (1980) determined that gray discoloration in fresh minced fish is probably due to release of skin melanins. The largest concentrations of melanins were measured at high machine pressure settings and at the far end of an auger type deboner.

Frozen minced flesh of three marine species became less red and more yellow during six months of storage and was somewhat darker when cooked (Nakayama and Yamamoto, 1977). Such yellowing and browning has been associated with lipid oxidation during frozen storage (Hansen, 1972). Fading of salmon carotenoid pigments during frozen storage also may be related to lipid oxidation (Botta et al., 1973).

A variety of approaches to correct color problems has been proposed. By adding phosphate to minced freshwater mullet, Baker et al. (1977) achieved less yellowing and improved color acceptability scores after frozen storage. Moledina et al. (1977) found that metal catalyzed reactions and perhaps other oxidative color changes in deboned flounder were minimized during frozen storage by the addition of 0.1% citric acid, 0.2% Kena (a phosphate mixture), 0.25% Na_2EDTA and either 0.1% ascorbic acid or 0.1% erythorbate. Acidification to pH 5.3 with citric acid and ascorbic acid lightened color without producing an objectionable chalky, granular appearance noticed at lower pH levels.

Diluting or masking color with starch or another white vegetable product (King, 1974), fat (Lanier and Thomas, 1978), titanium dioxide (Jauregui, 1978) or smoke (Bello and Pigott, 1979)

has been partially successful for certain products. Since much of the dark muscle is often close to the skin, several researchers suggested operating deboning machines at reduced belt pressure to allow pigment-rich tissue to pass with the wastes (Miyauchi et al., 1975; Patashnik et al., 1973; Steinberg, 1975).

White V-cuts or other light colored fillet trimmings can be combined with frames to dilute color (King, 1974). Keay (1979) recommended removal of the backbone dorsal to the visceral cavity or mixing light colored fish species with more pigmented species. Heme pigments are water soluble and can be washed from minced fish with cold water - a common practice in Japan (Okada et al., 1973; Kudo et al., 1973; Miyauchi 1972). Similarly, Beuchat (1973) observed that pigments were leached from skinned catfish when stored in ice. Washing also removes other proteins, principally sarcoplasmic (Setty et al., 1974). Bello and Pigott (1979) noted that washing is an expensive process and "should be avoided."

Prior to deboning Apolinario (1975) decolorized the skin of catfish by dipping them into a 5 percent sodium hydroxide solution for 30 seconds at 155 F. Jauregui (1978) removed fish skin with sodium hydroxide and partially oxidized the melanin in others by bleaching with sodium hypochlorite.

Patashnik et al. (1973) lightened apparent flesh color by forming an emulsion for use as a base in spreads. Small oil globules refract and reflect light from the product.

Flavor

Characteristic fresh fish flavors are caused by a wide variety of volatile compounds. Howgate (1976) reported improved flavor acceptability for cod when allowed to stand in ice for 1 to 4 days. Raja and Moorjani (1971) attributed such improvement primarily to the formation of inosine 5'monophosphate (IMP) resulting from the enzymatic deamination and dephosphorylation of adenosine triphosphate. The authors claimed that subsequent enzymatic degradation of IMP is responsible for "the loss of sweet flavour." Flavor improvement in shrimp during initial stages of iced storage may result from the release of free amino acids by native enzymes (Pedraja, 1970).

Off flavor development during storage of fresh fish is primarily a result of autolytic and microbial degradation which may be encouraged by mincing (Miller et al., 1972; Pedraja, 1970). Miller et al. (1972) identified up to 21 volatile ketone, aldehydes, alcohols and amines in ground canary rockfish muscle stored on ice. Production of dimethylamine in gadoid fish is primarily associated with muscles containing high concentrations of heme pigments (Castell et al., 1971). Dimethylamine formation in silver hake is enhanced by mincing (Lall et al., 1975). Intrinsic earthy or grassy flavors are often due to organic compounds absorbed by fish from their environment (Rippen, 1980).

Mechanical deboning may directly influence flavor. Crawford et al. (1972_b) reported an approximately 40 percent reduction in flavor panel scores for minced English sole and Dover sole when compared to intact fillets. The authors attributed the results to the mechanical

inclusion of volatile compounds associated with the skin in these species. No significant flavor changes as a result of mechanical deboning were observed with three other species. Inclusion of kidney material in minced fish may contribute off flavors (Dingle and Hines, 1975). Similarly, blood may carry a metallic flavor to minced fish products (Keay, 1979). Howgate (1976) suggested that cell rupture and subsequent release of intracellular materials may accelerate enzymatically induced flavor changes in mechanically deboned fish.

According to Field (1974) deboner heat may alter flavor of such raw materials as lamb breasts and broiler necks, especially when machines are adjusted for maximum yields.

Flavor of minced fish has been improved by mixing 1:1 with minced shrimp (Babbitt et al., 1974) or blending off flavored finfish with more acceptable finfish (Steinberg 1975). Patashnik (1974) masked intrinsic flavor of minced carp with onion or smoke flavorings. Many researchers proposed a post-deboning ice water wash to reduce odor and flavor of minced fish (Kudo et al., 1973; Miyauchi et al., 1975; Okada et al., 1973; Patashnik et al., 1973). In addition to removing undesirable compounds elevated moisture levels in the washed mince may dilute flavor (King, 1974). Cooking tends to drive off flavors and odors especially if fish are heated uncovered so that vapors escape (Beuchat, 1973). Phosphates may improve the flavor and aroma of minced fish (Baker et al., 1977). However, Manohar et al. (1973) found no consistent flavor preference for walleye or whitefish due to presence or absence of sodium tripolyphosphate.

Lipid Stability

Shelf life of frozen fishery products is often limited by the development of undesirable flavors and odors resulting from the oxidative deterioration of fats and oils. Lipid oxidation is a complex series of reactions initiated between unsaturated oils and oxygen and involves the formation of hydroperoxide intermediates and such terminal products as acids, aldehydes, ketones, alcohols and hydrocarbons. A review of this topic may be found in Labuza (1971).

Even small concentrations of the lower molecular weight compounds may contribute characteristic off flavors to fish. McGill et al. (1977) determined that hept-c4-enal and, to a lesser extent, two other aldehydes are the primary compounds responsible for cold storage flavor in cod. The authors reported an average flavor recognition threshold for hept-c4-enal of only 4×10^{-5}ppm in water which compares to an olfactory threshold of 1.5×10^{-3}ppm in oil (Labuza, 1971).

The type and concentration of end products created depends on fat content and other compositional characteristics of the fish. Lean fish (e.g. cod) generally oxidize to produce cardboard-like flavors, and fatty fish (e.g. mackerel) become typically rancid (Atkinson and Wessels, 1975; Cole and Keay, 1976). Saltwater mullet is highly susceptable to oxidative rancidity (Deng et al., 1977; Fischer and Deng, 1977). When analyzed by Finne et al. (1980) mullet lipids were composed of 34.5 percent polyunsaturated fatty acids, including 23.1 percent that contained 5 or 6 double bonds. A high level of fatty acid unsaturation and instability is common to many species of fish

(Stansby, 1973; Lindsay, 1975), especially marine species (Labuza, 1971).

As might be expected, tissues that are most susceptable to oxidation are usually highest in fat content such as belly flap, lateral line, dark (red) muscle and skin tissues (Stansby, 1973; Ke et al., 1977; Lee and Toledo, 1977).

Phospholipids were recognized as being relatively more important than triglycerides in the oxidation of cooked pork (Younathan and Watts, 1960). The frequently reported loss of phospholipids from fish during storage may reflect oxidative degradation directly or enzymatic hydrolysis (Mai and Kinsella, 1979_b; Wood et al., 1969). Due to the higher fat content of dark muscle compared to white muscle in sucker (6.2% versus 1.4%), dark muscle was found to contain 41.5% more phosphatidylcholine than white muscle (Mai and Kinsella, 1979_a). This was true even though white muscle contained more phosphatidylcholine when expressed on a percent of lipid basis.

The readily oxidizable nature of red muscle in fish is probably also related to heme pigment and iron content. Silberstein and Lillard (1978) demonstrated proportionately faster rates of oleic acid oxidation with increased heme protein concentration in mullet extracts. In a kinetic model system they also determined that metmyoglobin was a stronger catalyst than methemoglobin. This is important since mechanical deboning of mullet resulted in an increase of total heme pigment from 4.41 mg/g prior to deboning to 5.77 mg/g following debonding while the hemoglobin:myoglobin ratio rose from 0.57 to 1.20. These results suggest that although heme-catalyzed

oxidation may increase after mechanical deboning, the rate of increase due to the added hemoglobin should be less than would be expected if myoglobin was solely responsible for the increased pigment content.

Deng et al. (1978) demonstrated the reactive potential of ground dark muscle from mullet. Ascorbic acid oxidized at a much faster rate in red or mixed flesh than in all light colored flesh. This appeared to be a function of heme and iron content since red muscle and white muscle contained 15.97 ppm heme iron (40.75 ppm total iron) and 0.76 ppm heme iron (2.60 ppm total iron), respectively.

Lee et al. (1975) reported that hemeproteins are the predominant catalysts of lipid oxidation in mechanically deboned chicken. They found antioxidant as well as prooxidant characteristics of hemeproteins depending on relative concentration to substrate where a polyunsaturated fat:heme molar ratio of 500 maximized oxidation. Similar findings were observed by Hirano and Olcott (1971) in linoleate solutions. They suggested that at high concentrations, heme and hemeproteins may form appreciable quantities of oxidized porphyrin derivatives capable of acting as free radical scavengers. At low concentrations, the prooxidant effect was possibly due to their ability to decompose peroxides with the subsequent generation of free radicals for chain initiation.

Of several antioxidants investigated, Fischer and Deng (1977) achieved the most inhibition of lipid oxidation with cyanide which is known to bind strongly to heme proteins and stabilize them. This prompted their conclusion that heme iron is the major catalyst of

lipid oxidation in mullet flesh. The researchers also acknowledged the importance of nonheme iron arising from certain enzymes and nonenzyme sources such as ferritin. They reported evidence that 56 to 75 percent of total iron in mullet is nonheme iron. Silberstein and Lillard (1978) found that 2 to 14 percent of total iron was nonheme iron in mullet phosphate buffer extracts but suspected that their procedure was unable to account for all nonheme iron. In a cooked red meat model system nonheme iron possessed prooxidant activity but metmyoglobin demonstrated no activity (Love and Pearson, 1974).

Labuza's (1971) discussion of trace metal and heme catalyzed lipid oxidation theory can be summarized as follows: metals such as iron, copper, cobalt, nickel and manganese lower the activation energy required for initiation. This is possible by interaction with and subsequent decomposition of peroxides or by direct radical initiation with the substrate. The catalytic activity of a metal depends on its changing oxidation strate properties. Metals of greatest activity pass through a change of +2 to +3 where the +3 valence is most efficient at hydroperoxide decomposition. A slow regeneration of the +2 state favors the +3 form yet releases the metal from the peroxy complex (the metal is reduced) for reuse. The peroxyl radical (ROO.) produced becomes available to enter propagation reactions.

In heme pigments, ferrous or ferric iron is bound to the 4 nitrogens of a porphyrin ring as a planar chelate. The fifth coordination position is bound to the nitrogen of a histidine in the globin (globular protein) and the sixth position is available for complexing with oxygen or other moiety. During oxidation, a

hydroperoxide radical can bind to the sixth position despite possible steric hinderance from the globin. Because the electron orbital structure of iron is thermodynamically satisfied when complexed in this manner oxidation might be expected to terminate. However, Labuza (1971) suggested that due to the close proximity of the two electrostatically negative oxygens cleavage is likely if repulsed by the hemeprotein. The oxy-radical (RO.) formed is highly unstable and enters the propagation phase.

Unless closely controlled mechanical deboning promotes conditions conducive to lipid oxidation. Oxidation was accelerated when fish flesh was allowed to contact iron parts on a meat-bone separator (Lee and Toledo, 1977). Mai and Kinsella (1979_a) postulated that "... the deboning process and freezing may accentuate the release and mixing of prooxidants from dark muscle." Many researchers have noted the potential for increased lipid oxidation during and following mechanical deboning due to greater flesh surface area and incorporation of oxygen (Cole and Keay, 1976; Teeny and Miyauchi, 1972; Bremner, 1977; Patashnik et al., 1973).

Conversely, Steinberg (1975) pointed out that the minced form offers the opportunity for exposing more surface to antioxidants. Dawson et al. (1978) reported that mechanically deboned sucker was less vulnerable to lipid oxidation than ground eviscerated sucker (bones and skin included) or belly flaps, although more oxidizable than loin muscle.

Washing minced fish in water has been suggested to improve lipid stability as well as to improve color and flavor. Jauregui

(1978) and Miyauchi (1972) reported a reduction in lipid content following washing and, as previously discussed, hematins and other water soluble constituents are at least partially removed during the washing process which probably contributes to slower oxidation rate (Steinberg, 1975). Washed minced fish is commonly dewatered to a water content 3 to 10 percent higher than prewash flesh (Miyauchi et al., 1975; Patashnik, 1974; Patashnik et al., 1976) which may minimize contact of reactants with substrate (Labuza, 1971).

Texture

The act of mechanical deboning results in a loss of muscle integrity. Webb et al. (1976) believed that texture loss was associated with the shearing of myofibrillar proteins and their subsequent denaturation. However, the view is not substantiated by research. In a study by Wong and Yamamoto (1978) a direct relationship existed between fish texture and machine perforation size. This was attributed to an elevated moisture content resulting from increased compression observed in flesh processed through small holes. Texture may range from "mushy" at a high water content to "rubbery" at a lower water content (Lanier et al., 1980). Patashnik (1974) reported improved texture for products derived from carp when deboned with a machine equipped with 7mm perforations compared to 1.4mm perforations.

Many factors affect the texture of minced fish products including species, maturity, season, location and freshness (Miyauchi

et al., 1973), and temperature, pH and ionic strength (Lanier et al., 1980; Moledina et al., 1977; Okada et al., 1973). Release of proteolytic enzymes during the mincing operation has been implicated in the softening of deboned fish. Cathepsins, originating from lysosomes, are most active in an acid environment at moderate temperatures, while alkaline proteases of cytoplasmic origin are functional at alkaline pH and at -20°C to 60°C (Lin et al. 1980; Lin and Lanier, 1980). The enzymes are 200 to 1500 times more active in kidney tissue than in muscle and represent the major "contaminant" in minced croaker (Lin et al., 1980).

Protein denaturation is manifested as a loss of salt extractability and water holding capacity with concomitant toughening. The toughening commonly associated with frozen fish is probably due more to hydrogen bonding and hydrophobic interactions between polypeptides than to disulfide linkages (Iwata and Okada, 1971). The protein conformational changes required to encourage such bonding may be due to interaction with free fatty acids (Anderson and Favest, 1969), products of lipid oxidation such as malonaldehyde (Anderson, 1970; Jarenback and Liljemark, 1975) and dimethylamine or formaldehyde in gadoid species (Dingle et al., 1977; Babbitt et al., 1972).

Washing minced fish in water is known to improve binding properties and water holding capacity (Miyauchi et al. 1973; Kudo et al., 1973 Miyauchi et al., 1975). This effect is usually attributed to a relative increase in myofibrillar proteins due to loss of water soluble proteins (Okada et al., 1973; Patashnik et al. 1976) and to removal of proteases (Okada et al. 1973; Lanier et al., 1980).

The latter study also suggested that water extraction of prooxidants may aid the retention of protein functionality.

Microbiology

Freshly caught whole fish possess bacteria in gills, slime and digestive tract and are essentially free of organisms in the tissues (Reay and Shewan, 1949; Hunter, 1933). These bacteria vary in composition depending on location. According to Shewan (1962) fish found in tropical waters carry significant quantities of mesophilic varieties while fish from northern waters are associated with over 95 percent psychrotrophs. Researchers have related spoilage of iced or refrigerated fish primarily to psychrotrophic varieties, particularly species of Pseudomonas and Achromobacter (Reay and Shewan, 1949; Castell and Anderson, 1948). Freshwater fish are spoiled by a somewhat different microflora than marine species and reportedly maintain quality longer than saltwater fish (Nair et al., 1971, 1974).

The penetration of surface bacteria into the flesh is greatly assisted by mechanical deboning. Raccach and Baker (1978) found a ten-fold increase in bacteria count following deboning but acknowledged a possible deficiency in the sampling method employed. They suggested that mincing may release nutrients, such as amino acids and vitamins, that improve the conditions for microbial growth. Pedraja (1970) pointed out that bacteria are incapable of attacking intact proteins and must be assisted by native enzymes or mechanical rupture.

CHAPTER III

EXPERIMENTAL

Materials

Procurement of Fish

All carp used in this study were caught commercially by trap net or gill net from Saginaw Bay, Lake Huron. December and January batches were purchased from Beardsley Fish Co., Standish, Michigan and all other batches from Bay Port Fish Co., Bay Port, Michigan. Carp were boxed in ice 0-5 days at time of purchase, were reiced and transported without additional refrigeration to the Michigan State University Meat Laboratory. When required the fish were topped with additional ice but always stored at 2°C and processed within 2 days. Most fish weighed in the range of 2.3-6.7 kg.

Fish Preparation and Mechanical Deboner Operation

Carp were weighed as a group when yield data were desired and manually headed, gutted and split dorsoventrally parallel to the backbone. The split carp were washed under cool running tap water, using a hand brush to facilitate removal of kidney material. They were reweighed when needed and immediately layered with crushed ice until deboned.

The mechanical deboner was a Bibun model SDX13 (Bibun Co., Fukuyama Kiroshima, Japan) belt type machine equipped with a 3mm

perforation size drum. The dressed carp were passed through the machine flesh side to the drum and the minced flesh was recovered with a plastic lug. On one occasion flesh temperatures were recorded prior to deboning, at the drum mouth and upon completion of the deboning operation. The minced fish was covered with plastic film and stored at 2°C until analyzed the same day or packaged and frozen for later analysis depending on the parameter to be tested. A preliminary trial was conducted to determine the need for passing the minced flesh through the machine a second time to remove remaining bone and scale residue. In this instance first and second pass samples were collected for yield, proximate composition, bone and scale, Hunter color difference, shear press shear force, TBA and total plate count data.

Treatment Preparation

Antioxidants

A procedural flow diagram for preparation of control and antioxidant treatments is presented in Figure 2. Freshly minced carp flesh was mixed with a stainless steel paddle and combined with 0.02% w/v Tenox 2 (based on antioxidant content, not carrier), 0.18% w/v Freezgard (formula FP-88E) or no antioxidant in a Hobart paddle type mixer (Hobart Corp., Troy, Ohio) set at low speed for 3 minutes. Tenox 2 (Eastman Chemical Products, Inc., Kingsport, Tenn.) is a mixture of 20% butylated hydroxyanisole, 6% propyl gallate, 4% citric acid and 70% propylene glycol. Freezgard (Stauffer Chemical Co., Westport, Conn.) is a blend of sodium hexametaphosphate, NaCl and sodium erythorbate in undisclosed proportions.

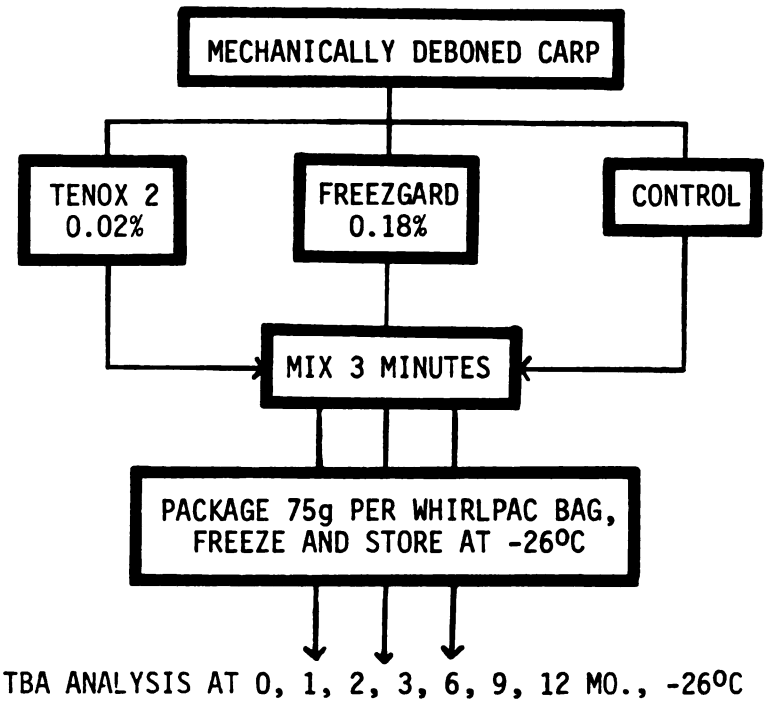

Figure 2. Preparation of antioxidant treatments.

The control and two treatments were packaged in whirlpac bags (Nasco), approximately 75g per bag with free air removed in so far as possible, then identified, frozen and stored at -26°C. Samples were subsequently analyzed for TBA value at months 0,1,2,3,6,9 and 12, where month 0 was frozen for 1-3 days prior to analysis. The procedures were replicated 3 times using a different batch of fish for each replication.

Corrective Treatments and Parameters Investigated

Four water wash treatments were developed based on methods described by Patashnik et al. (1976) and Kudo et al. (1973) for plain water washing, defatting and dewatering of minced fish flesh. See Figure 3 for a flow diagram of the procedure. Freshly deboned carp was washed in ice water at a 4:1 weight ratio of ice water to fish. The mixture was manually agitated with a stainless steel paddle for 3 minutes, allowed to settle for 1 minute and the coagulated fat was skimmed off the surface. The supernatant was slowly poured off through a fiberglass window screen that was tied over a plastic tub for collecting wash water. The procedure was repeated with a 3:1 ice water to fish ratio. Following the second fat skimming step the entire slurry was poured onto the screen, then covered and dewatered by gravity 2-4 hours at 2°C. The dewatering process was encouraged by gentle agitation at 20 minute intervals. The washed mince was considered to be sufficiently dewatered by a subjective evaluation corresponding to a yield ranging from 78 to 82% of original whole mince weight.

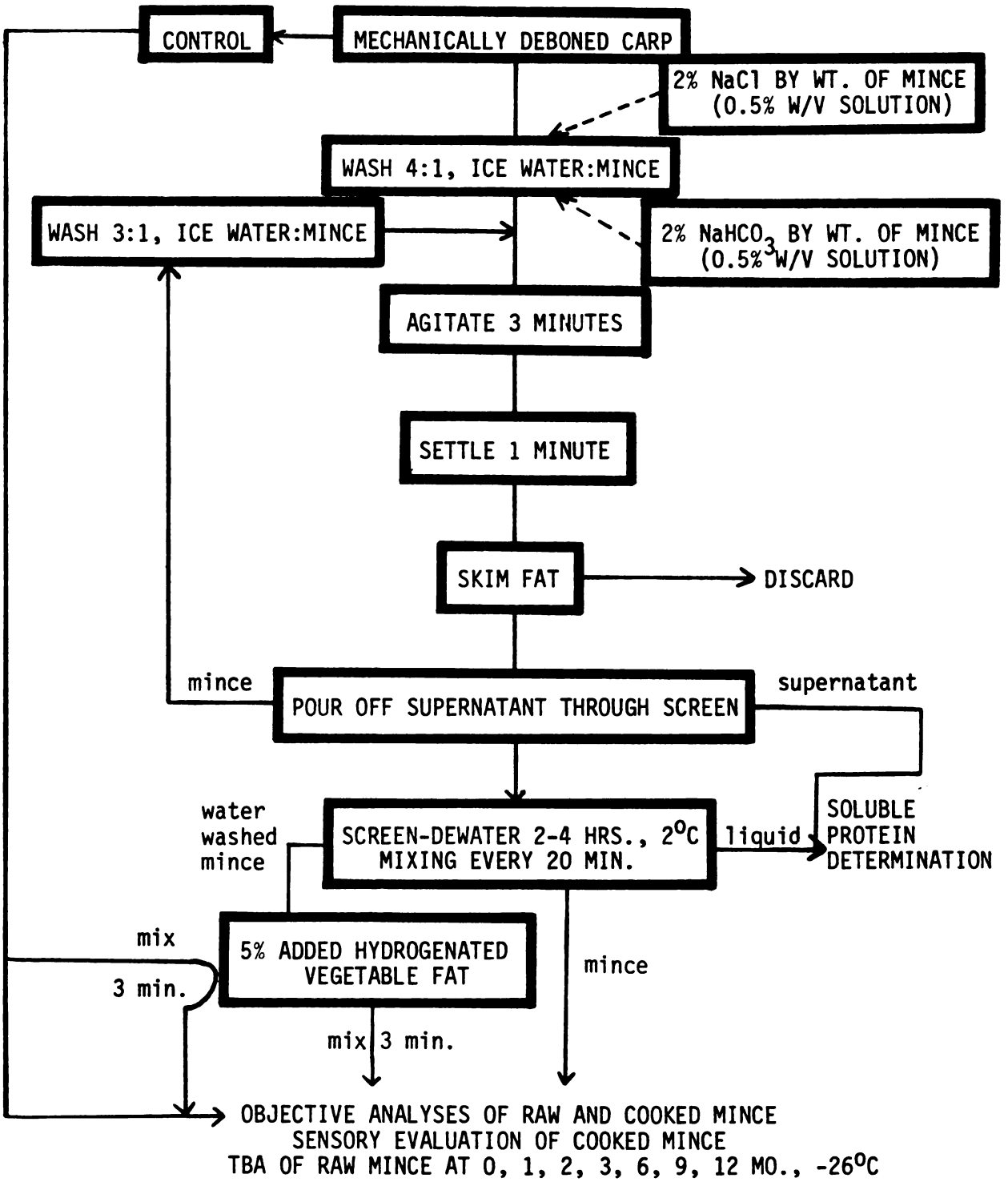

Figure 3. Preparation of four washed minced carp treatments.

Sodium chloride and sodium bicarbonate treatments were prepared as above except that either 2% NaCl or $NaHCO_3$ by weight of mince was predissolved and added to the primary wash (0.5% w/v solution). The second wash was a plain ice water wash only followed by dewatering to 72-78% of prewash weight. The fourth treatment consisted of adding 5% w/v hydrogenated vegetable fat to plain water washed mince. The fat was composed of hydrogenated soybean, palm and cottonseed oils which did not contain antioxidants (Holsum Foods, Waukesha, Wisc.). The fish was added slowly to the vegetable fat while mixing at slow speed with a Hobart paddle type mixer, mixing a total of 3 minutes. A corresponding control was similarly mixed 3 minutes. All other wash treatments corresponded to an unmixed control. Water wash and control samples were packaged in polyethelene freezer bags and stored at -26°C for later comparison of bone and scale content (4 replicates); NaCl and control samples for NaCl determination (3 replicates). The wash waters were paddle agitated, then sampled and frozen in identified capped vials for subsequent analysis for Biuret protein.

A hydrogen peroxide bleaching procedure was prepared following the method of James and McCrudden (1976), Figure 4. Freshly deboned carp was mixed with 1.0% sodium tripolyphosphate and 0.85% H_2O_2 (by weight of mince) while mixing at medium speed in a Hobart paddle type mixer. The pH was adjusted to 10.5 with 5N NaOH as measured by a model 10 Corning pH meter (Corning Scientific Instruments, Medfield, Mass.) fitted with a Sargent glass pH electrode (S-30072-15, Sargent-Welch Scientific Co., Detroit, Mich.). After 3 minutes of mixing the mince was allowed to stand 12 minutes at 16°C, then adjusted to pH

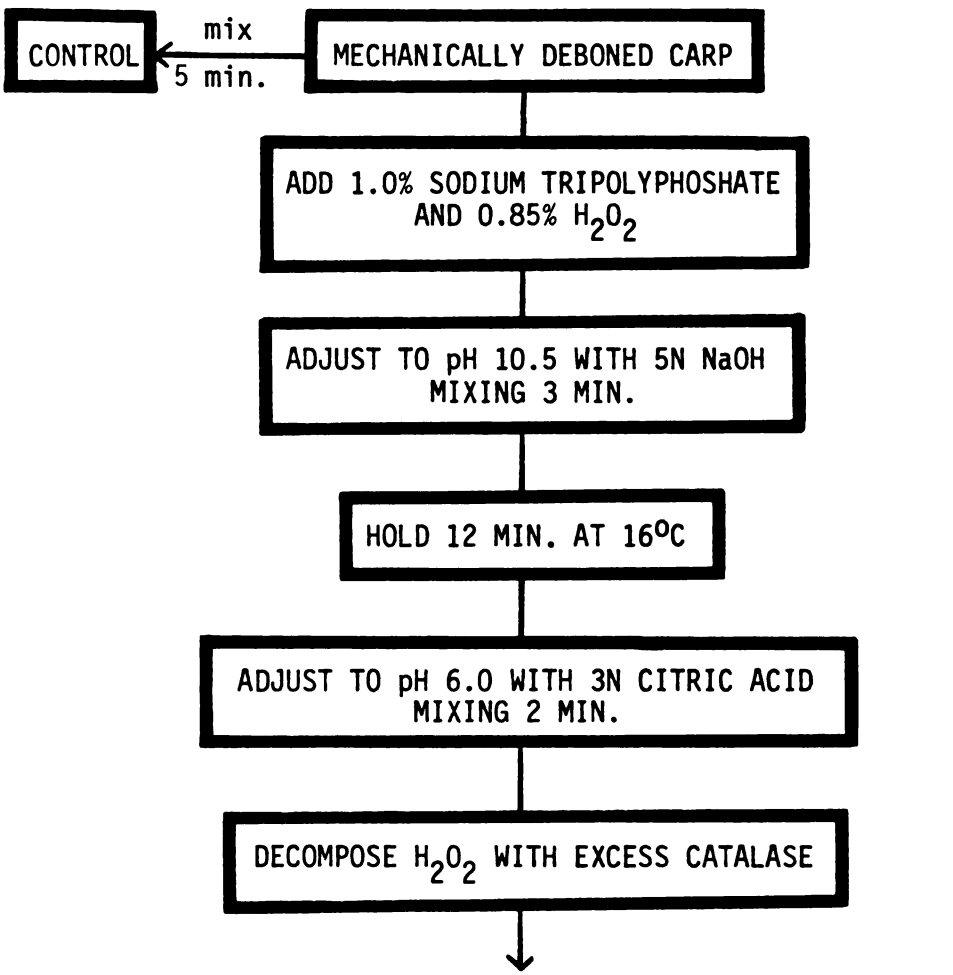

Figure 4. Preparation of hydrogen peroxide treatment.

6.0 with 3N citric acid while mixing an additional 2 minutes. Predissolved bovine liver catalase (Sigma Chemical Co., St. Louis, Mo.) was added in excess near the end of mixing to decompose residual H_2O_2. A control was similarly mixed 5 minutes.

An hexane extracted treatment was prepared as diagrammed in Figure 5. Cold hexane was added 2:1 (Hexane: mince, by weight) and manually stirred with a glass rod for 2 minutes. The mixture was filtered for approximately 5 minutes through four layers of cheesecloth with a Buchner funnel attached to a vacuum flask and water aspirator. The mince was then spread in a thin layer (about 1.5cm) on cheesecloth suspended over a glass baking dish to assist in removal of hexane. It was transferred to a vacuum chamber (model 5831, National Appliance Co., Portland, Oregon) and evacuated to 30 in.hg vacuum with a vacuum pump (model 1405, Sargent-Welch Scientific Co.) for 3 hours at 16°C. The mince was mixed at 45 minute intervals during the vacuum step. Due to equipment capacity limitations a maximum of 600g of mince (pretreatment weight) could be treated at one time and usually two batches were prepared per replication. Hydrogenated vegetable fat (previously described) was added to the extracted mince at the rate of 5% of the pretreatment mince weight by adding fish to the fat while mixing one minute at slow speed in a Hobart paddle type mixer. A control was similarly mixed for 1 minute.

All "corrective" treatments were packaged, frozen, stored at -26°C and subjected to TBA analyses according to the procedure previously described for antioxidant treatments. Samples were also

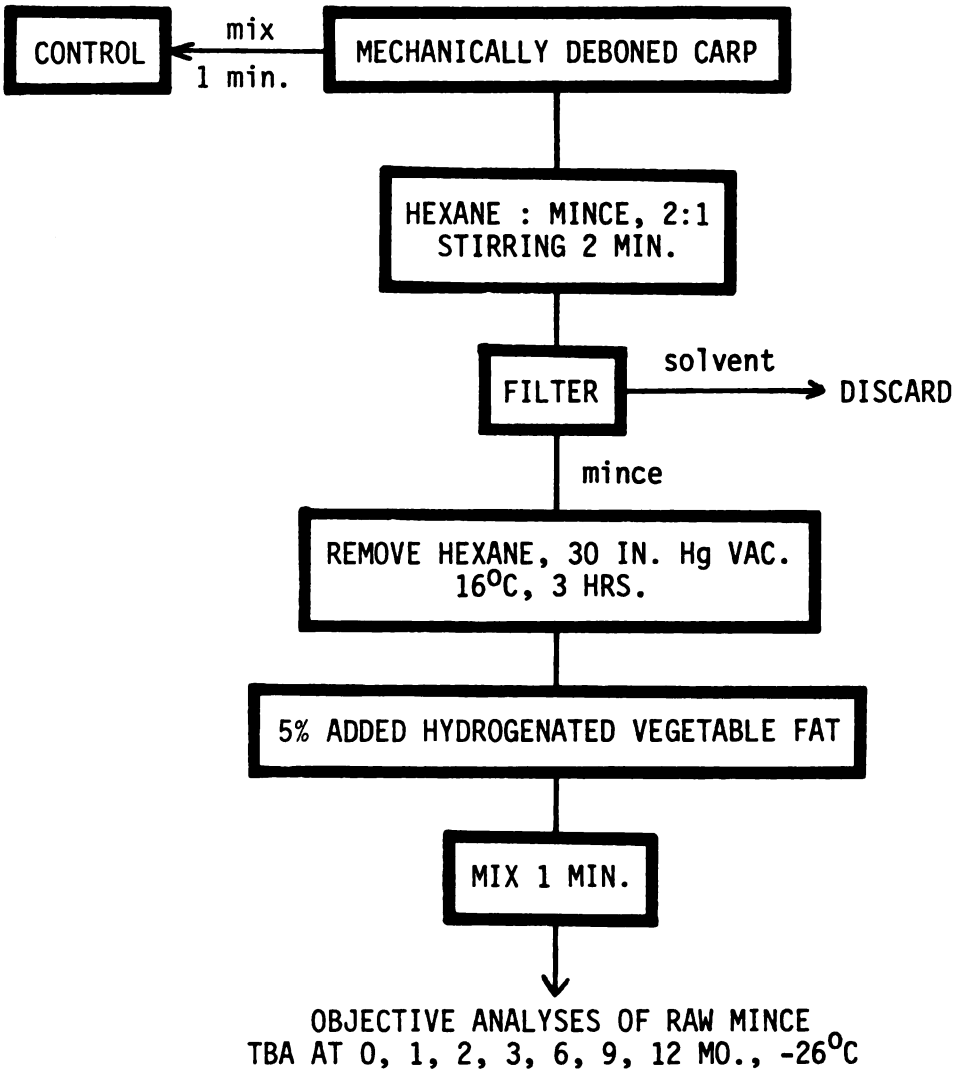

Figure 5. Preparation of hexane extracted treatment.

Whirlpac or vacuum packaged and stored at -26°C up to 3 days for TBA analysis or longer when destined for proximate composition. Fresh samples, held covered up to 4 hours at 2°C were analyzed for total plate count, TBA, Hunter color difference (L,a,b), shear force resistance, and pH. Other samples were vacuum packaged in barrier bags, frozen and stored at -26°C for 3 weeks prior to sensory evaluation or for 10 weeks prior to determination of heme pigment and cooking effects.

Methods

Microbial Analysis

Minced carp flesh was analyzed for total plate count (aerobic and facultative microorganisms) by the procedure of Frazier et al. (1968). Samples were removed from appropriate lots of mince and stored on stainless steel plates covered with plastic film in a 2°C cooler until plated (10 min. to 3 hrs.). The order that samples were plated was varied so that treatments were exposed to approximately the same average holding time.

Approximately 11 grams of sample was accurately weighed to two decimal places, blended 2 minutes in a single speed Waring blender (Waring Products Corp., Winsted, Conn.) with 99ml of 0.0003M sterile phosphate buffer. Appropriate serial dilutions were prepared by pipetting 1.0ml of aliquot from previous mixed dilutions into 99ml sterile phosphate buffer blanks. Samples were plated in triplicate with 2 plates per dilution. Sterile plate count agar was held at 45°C prior to pouring onto the plates which were then swirled in a figure 8 pattern and allowed to cool. Plates were inverted, covered

loosely with aluminum foil and incubated 72 hours at room temperature (approximately 25°C). Plates containing 30-300 colonies were counted and total plate count recorded as microorganisms/g. All equipment and materials used in this procedure were autoclave sterilized except petri plates which were sterilized by the manufacturer (Miles Laboratories, Inc., Naperville, Ill.).

Bone and Scale

Bone and scale content was determined by a method modified from one proposed by Wong and Yamamoto (1974). Triplicate 100g samples of minced carp were stirred at room temperature (25°C) overnight in 2000ml of 3M urea and 0.02 N NaOH on a Multi-Magnestir (Lab-line Instruments Inc., Melrose Park, Ill.). The contents were filtered through a 425 micron sieve and the residue resuspended in 50ml of the urea-NaOH solution for 4 hours with a magnetic stirrer. Bone and scale fragments were allowed to settle for 30 minutes. The liquid was carefully poured off through a 425 micron sieve and the remaining residue was washed several times with distilled water, then dried in a 105°C oven for several hours. Due to the presence of fatty material the residue was stirred with petroleum ether and filtered through dried and tared Watman No. 1 filter papter. After an initial period of air drying the samples were oven dried at 105°C for 12 hours, cooled in a desiccator and weighed. The cleaned residue weight (g) was recorded as percent bone and scale.

Moisture

The moisture content of minced carp was determined by the A.O.A.C. (1965) procedure (page 3, paragraph 23.003). Each of four replicate subsamples of about 5 grams were weighed accurately into a tared and dried aluminum dish and then dried overnight (approximately 18 hours) in a convection oven at 105°C. The dried sample was transferred to a desiccator until cool and accurately weighed. Weight loss was recorded as moisture and expressed as percentage of original weight.

Fat

The extractable lipid fraction was determined by the Goldfisch extraction method described by the A.O.A.C. (1965) procedure (page 3, paragraph 23.005). The aluminum dishes containing the samples previously analyzed for moisture were folded, placed into a porous porcelain thimble and extracted into a previously dried and tared beaker with anhydrous ether for 4 hours on the Goldfisch apparatus (Laboratory Construction Co., St. Louis, Mo.). The extract was dried for a minimum of 4 hours in a 105°C convection oven, then cooled in a desiccator and accurately weighed. Fat content was recorded as a percentage of fresh fish weight.

Protein

Protein content was calculated from the micro kjeldahl nitrogen determination of the A.O.A.C. (1965, page 3, paragraph 23.009). Approximately 0.5g of minced carp were accurately weighed in triplicate into micro kjeldahl digestion flasks. To this was added 1ml 10%

$CuSO_4$, 1g anhydrous Na_2SO_4, 7ml H_2SO_4 and a few glass beads. The flasks were heated on a rotary kjeldahl digestion apparatus until the solution became transparent green, then digested an additional 30 minutes. The flasks were allowed to cool prior to adding 15 ml deionized water.

Ten ml 2% boric acid and 3 drops Bromcresol green were added to erlenmeyer flasks which were mounted on distillation units such that the outlet tubes were positioned below the surface of the boric acid. The digestion flasks were attached to form a closed system and an excess of 44% NaOH solution was added to the sample flasks. Steam was immediately let into the system and allowed to distill for 7 minutes. The erlenmeyer flasks were then lowered and distillation was continued for another 3 minutes. The boric acid containing recovered ammonia was titrated to the Bromcresol endpoint with standard H_2SO_4. Percent protein was calculated by the formula:

$$\% \text{ protein} = \frac{(\text{ml } H_2SO_4)\ (\text{normality of } H_2SO_4)\ (14)\ (6.25)\ (100)}{\text{weight of sample, mg}}$$

Where, 14 = molecular weight of nitrogen
6.25 = 100/16% nitrogen in protein (assumed).

Thibarbituric Acid Test (TBA)

TBA numbers were determined by the method of Tarladgis et al. (1960). Triplicate 10g samples of minced carp were homogenized for one minute with 50ml distilled water in a Virtis homogenizer (Model 6-105-AF, Virtis Co., Gardiner, N.Y.) set at medium speed. The slurry for each replicate was transferred to a 500ml distillation flask with 47.5ml distilled water and the pH was adjusted to 1.5 with

2.5ml 4 N HCl. Several glass beads and a few drops of Antifoam A (Dow Corning Corp., Midland, MI) were also added to the flask. The flask was connected to a 30.5cm distilling tube followed by a condenser unit. The sample mixture was heated and allowed to distill until the first 50 ml of distillate was collected.

Two 5ml aliquots of the distillate were pipetted to test tubes to which were added 5ml of 0.02M thiobarbituric acid in 90% redistilled glacial acetic acid. The tubes were capped, agitated, and heated in a boiling water bath for 35 minutes and then cooled in cold water for 10 minutes. Color complex development was measured by absorbance at 538nm against a TBA reagent blank containing 5ml distilled water instead of distillate. TBA value was calculated by multiplying mean absorbance by the distillation constant 7.8, and was recorded as mg malonaldehyde/1000g sample.

Shear Force

Resistance of raw or cooked minced carp to shear was determined with a Kramer shear press (Model TR 3 Texurecorder, Food Technology Corp., Rockville, Md.) characterized by a ram descention speed of 0.52cm/sec. and fitted with a CS-1 multiblade shear-compression cell and a 3000lb compression ring. Triplicate 100g (approximately) raw samples were weighed, spread across the bottom of the cell and sheared. Triplicate cooked samples were cut from portions with a template to fit the cell, then weighed (approximately 75g) and sheared. Total force (compression plus shear) was recorded as lb shear force/g sample and was calculated from chart peak height by the formula:

$$\text{lb/g force} = \frac{(3000\text{lb ring}) \times \left(\frac{1}{\text{range}}\right) \times \left(\frac{\text{peak height}}{100}\right)}{\text{sample wt., g}}$$

Where, range = chart recorder sensitivity setting.

Color

Color was characterized by a model D 25-2 Hunterlab color difference meter (Hunter Associates Laboratory, Inc., Fairfax, Va.) which was standardized against a white standard. Two replicate 100g samples were each measured twice for "L", "a" and "b" values and each replicate was rotated 45° between determinations. These values represent reflectance ranges from black to white (0 to 100, L value), green to red (-a to +a) and blue to yellow (-b to +b). The samples were usually taken from the Kramer shear press after shearing and were uniformly pressed into a transparent dish to minimize textural differences.

Heme pigments

The concentration of total heme pigments was determined by the combined methods of Rickansrud and Hendrickson (1967) and Fleming et al. (1960). Duplicate 50g samples of minced carp were blended 3 minutes with 100ml of 0.01N cold (4°C) acetate buffer adjusted to pH 4.5. They were centrifuged at 2000 x g for 15 minutes, the supernatant then filtered through 4 layers of cheese cloth with the aid of a Buchner funnel, vacuum flask and water aspirator. The funnel was cleared with approximately 75ml of the cold acetate buffer and the supernatant contrifuged again at 2000xg for 15 minutes. This final supernatant was filtered through Watman No 1 filter paper and brought to volume with distilled water in a 250ml volumetric flask. To 20ml

of aliquot was added 2ml $K_3Fe(CN)_6$ reagent (0.9m Mole/100ml) and 3ml of KCN reagent (8.0m Mole/100ml) to stabilize pigments. Absorbance was read at 540nm and converted to heme pigment content by:

$$\text{Mg total heme pigment/g fish} = \frac{\text{absorbance}}{11{,}300} \quad \frac{17{,}000 \times 0.3 \times 1000}{\text{sample wt., g}}$$

Where, 11,300 = molar extinction coefficent
17,000 = estimated equivalent weight of pigments
0.3 = volume of extract in liters

Sodium Chloride

Chloride as NaCl was determined in control and NaCl washed, minced carp by the Volhard titration procedure (Stine, 1978). Triplicate 10g samples were accurately weighed and transferred to erlenmeyer flasks. Twenty ml of 0.1 N $AgNO_3$ (standardized) followed by 20ml of nitric acid were added to each flask and then boiled under a hood until dissolved. Digestion of organic matter was assisted by adding concentrated $KMnO_4$ as required. The solution was boiled until nearly colorless, then approximately 25ml distilled water was added and boiling continued for another 5 minutes. The flasks were cooled and distilled water was added making to about 100ml. A small quantity of acetone was poured down the inside wall of each flask to prevent resolution of precipitated AgCl. The flasks received approximately 1ml $Fe(NH_4)(SO_4)_2$ indicator solution acidified with nitric acid. They were then titrated to a permanent light brick red endpoint with standardized 0.1N NH_4SCN and calculated for NaCl content with the following formula:

$$\text{Percent NaCl} = \frac{(\text{Blank} - NH_4SCN, \text{ ml})\ (\text{normality of } NH_4SCN)\ (0.0584)(100)}{\text{Sample wt., g}}$$

Where, 0.0584 = milliequivalent wt. of NaCl
Blank = theoretical ml of NH_4SCN needed to react with all $AgNO_3$ (= 20ml if used 20ml 0.1N $AgNO_3$).

Soluble Protein

Solublized protein removed in wash treatment waters was determined by the Biuret method of Goa (1953). Duplicate 0.1 or 0.2ml samples of wash water were brought to 2ml volume. Two ml of 6% NaOH solution was added, then the samples were mixed prior to receiving 0.2ml Biuret reagent. After mixing again and allowed to stand for 15 minutes, absorbance was measured at 540nm.

A standard curve was prepared with bovine serum albumin made to a concentration of 125mg BSA/25ml. Aliquots of the standard containing a range of 0-5mg protein were measured by the Biuret procedure and the constant, mg protein/absorbance, was determined. Protein content of the wash waters was then calculated from the formula:

$$\text{mg protein/ml} = \left(\frac{\text{absorbance of sample - absorbance of blank}}{\text{mg protein/absorbance}}\right) \Bigg/ \text{sample, ml}$$

Buiret reagent is made by the following procedure:

1. Dissolve 173g Na citrate and 100g Na_2CO_3 in 500ml distilled water.
2. Dissolve 17.3g $CuSO_4 \cdot 5H_2O$ in 100ml distilled water.
3. Add solutions together and make to 1000ml - discard if reddish precipitate forms.

Cooking of Fish

A control and 3 replicates of "corrective" treatments were prepared from one batch of fish and then cooked for 40 minutes at 175°C in covered aluminum baking dishes (351g/dish) with a convection oven (model CV912, Crimsco, Inc.). Due to residual solvent the hexane extracted treatment was not cooked. After removing from the oven the dishes were set on one end for 5 minutes and then on the opposite end for another 5 minutes to allow cooked out fluids to escape and the fish to cool. Triplicate samples were cut, weighed and sheared, then analyzed for Hunter L, a and b values. Yield of cooked flesh (percent of precook weight) was determined only once.

Sensory Evaluation

Minced carp was mixed as one large uniform batch and prepared into the 6 "corrective" treatments plus a control. The hexane extracted mince was not utilized for taste panel study due to residual solvent. The minces were vacuum packaged, frozen and stored at -26°C for 10 weeks prior to sensory evaluation.

Judge Selection

Twenty-three prospective taste panelists were screened for their ability to detect differences in flavor intensity of minced carp. Since two research projects had similar panel requirements, a system was employed to develop one screened panel for both sets of evaluations. A standard fish ball formulation was used to make two batches - one contained water washed minced carp and one contained unwashed minced carp. The fat content was adjusted to yield products

with similar proximate compositions. The balls were deep fat fried at 165°C to a uniform brown color, however some distinction between treatments was possible based on outside and inside color.

Eight successive triangle tests were given each judge - 4 tests with 2 samples containing water washed mince against one sample with unwashed mince and 4 vice versa (see Appendix B.1 for score sheet). The order of presentation of the 2 arrangements was determined randomly from a table of arrangements. The number of incorrect decisions (inability to detect the odd sample) was totaled for each panelist.

Prior to evaluation it was tentatively decided that 4 incorrect judgments was acceptable but that 5 or more would be grounds for rejection. This was based on the test characteristic, $p = 1/3$ by chance only. The criterion proved workable since after plotting the results, a bimodal distribution occurred among the judges with the second peak beginning at 5 or more errors (Figure 6). Five panelists were rejected on this basis, leaving 18 screened judges who were invited to attend 3 subsequent panel sessions. Attendance was reasonably good with 15 judges participating in the first session and 17 judges attending each of the last 2 sessions.

Preparation and Evaluation of Treatments

Fish treatments were evaluated without adding seasonings or other ingredients. Approximately 15g samples were portioned into individual foil pouches without added seasonings or other

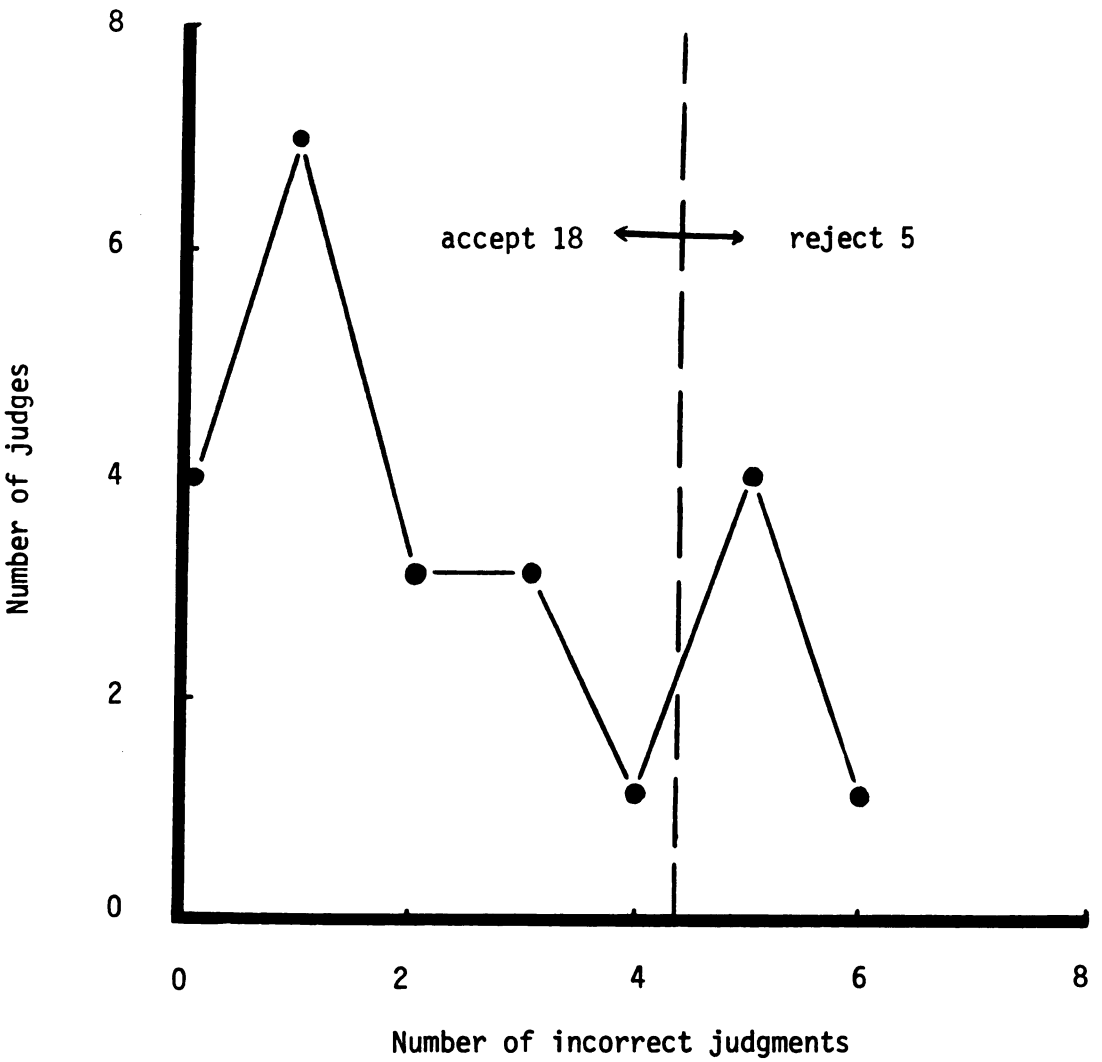

Figure 6. Performance of prospective judges during screening trial, $p \geq \frac{1}{2}$ to accept.

ingredients and were baked in a convection oven at 175°C for 15 minutes. They were then held at 93°C until just prior to serving.

Each treatment was assigned a list of five 3-digit code numbers between 100 and 1000 from a random number table before every tasting session. The order that samples were placed around the paper serving plates was randomized differently for each panelist. The samples were served in individual aluminum weighing dishes which were coded with numbered tape tabs.

Evaluation sessions were held at 10:00 a.m. or 3:00 p.m. in the taste panel facilities of the Food Science Building, Michigan State University, where 8 fluorescent lit partitioned booths were used adjacent to the preparation kitchen. Judges were supplied with water, plastic forks, napkins, spittle cups, pencils, a set of 4 score sheets and a plate of samples.

Judges were informed to rank the six samples in the following order of stated categories: color intensity (dark to light), color preference (most preferred to least preferred), flavor intensity (strongest to weakest), flavor preference (most preferred to least preferred), texture (firmest to softest), texture preference (most preferred to least preferred) and overall preference (like best to like least). The score sheets explained that the 6 samples could be evaluated as often as needed and in any order. Ranking as a means of sensory evaluation and its statistical analysis are discussed by Kahan et al. (1973).

Statistical Analysis

Analysis of variance tables were generated by submitting raw coded data into the genstat packaged program interfaced with the main computer system at Michigan State University. Analysis of variance for 2 way classifications containing unequal replications involved computer selection of best fit data by iterative least squares analysis which resulted in the loss of one or more degrees of freedom. This condition occurred in the lipid stability storage studies due to failure to determine month 0 TBA values for the first of 3 replications in the 2 antioxidant treatments and to storage of 3 replications of control, plain water wash, NaCl wash and $NaHCO_3$ wash treatments compared to 2 replications of wash plus fat, H_2O_2 and hexane extracted treatments.

Where indicated by significant variance ratios, means were compared by Tukey's procedure (Steel and Torrie, 1960). Following the antioxidant storage study, the mean treatment TBA values were compared to the control by Dunnett's procedure (Steel and Torrie, 1960) as well as being compared to the control and each other with Tukey's method.

Possible correlations among proximate composition, bone and scale, color, shear force, TBA and total plate count data following the deboning operation were investigated by applying simple correlation coefficients and consulting a table of significant r values (Snedecor and Cochran, 1967).

Sensory determined mean ranks were analyzed by the method described by Kahan et al. (1973) for ranked data. The procedure

is based on F statistics which when significant permit comparisons by least significant difference. Since the L.S.D. method is relatively liberal at detecting differences only means different at the 1% level were reported as significant.

Statistical analyses were performed on actual measurements when possible or practical. However, since not all treatments were processed on the same day and mixed (agitated) treatments corresponded with mixed controls, not all "corrective" treatments could be directly compared. Consequently, values were expressed as percent of corresponding control or simple difference from corresponding control depending on the data analyzed. Comparing arithmatic differences also helped to reduce replication variance due to intrinsic characteristics unique to each batch of fish.

CHAPTER IV

RESULTS AND DISCUSSION

Part I: Deboner Efficiency and Characteristics of Mechanically Deboned Carp

Based on research by Zapata (1978) a preliminary deboning trial was conducted to indicate the need for passing minced carp through the mechanical deboner a second time. Although an additional 30% reduction in bone and scale residue was realized after a second deboning pass, the first pass determination of bone and scale content was low, 0.034% (Table 2). This compares to 0.15% bone and scale for sucker following two passes through a deboner equipped with 5mm holes (Zapata, 1980).

Table 2 also indicates that a loss of textural integrity (reduced shear resistance) resulted from the second pass, as well as a possible increase in lipid oxidation (elevated TBA value). Due to these preliminary findings a second deboning pass was not considered valuable for carp and no further investigation was attempted. During a later deboning trial, temperatures were recorded as follows: dressed carp prior to deboning, 1.1^{o}C; minced carp at machine drum exit, 4.4^{o}C; maximum temperature of mince before returning to cooler, 8.9^{o}C. It is reasonable that elevated temperatures encountered during a two pass process may accelerate proteolysis and lipid oxidation since reaction rates for both are temperature dependent.

TABLE 2.--Effect of processing minced carp by one or two passes through mechanical deboner, n = 1 replication.

	Whole(kg)	Dressed(kg)	Yield(%)[1]	First Pass	Yield(%)[2]	Second Pass	Yield(%)[3]	Net Yield(%)
Weights and yields	48.1	29.7	62	20.8	70	19.9	96	41
Moisture(%)				68.43		70.13		
Fat(%)				15.46		14.81		
Protein(%)				13.42		13.47		
Bone & scale(%)				0.034		0.024		
Color L				40.9		42.5		
Color a				10.1		9.5		
Color b				11.3		11.8		
Texture, shear force(kg/g)				0.305		0.171		
TBA number				2.19		2.71		
Plate count, (organisms/g)				1.5×10^4		1.8×10^4		

[1]Percent of dressed weight.

[2]Percent of first pass weight.

[3]Percent of whole fish weight.

A reduction of particle size also may contribute to decreased shear resistance and may encourage lipid oxidation by increasing surface exposure to oxygen or prooxidants.

An apparent increase in moisture content of second pass flesh is consistent with the findings of Wong et al. (1978) who reported more inhibition in the passage of solids than water when forced through small perforations. A slight whitening effect (larger Hunter L value) following the second pass suggests a partial emulsification of lipids, a characteristic more fully realized in finely chopped fishery products (Patashnik et al., 1973).

Yields of mechanically deboned carp are reported in Table 3. The values of 61% and 42% for yield from dressed carp and round carp, respectively, are below those figures determined for sucker (68% and 48%, Dawood; 1979). This may reflect the frequent occurrence of gravid females and the fact that carp possess thick skins and a heavy bone and scale structure. Angel and Baker (1977) reported low fillet yields for carp and an average skin weight of 8.4 percent of headed and gutted weight compared to 4.9 percent skin for headed and gutted hake. Minced carp was found to be relatively high in fat and low in moisture and protein compared to that of many other species (Merritt, 1974), Table 4. These percentages may be more characteristic of carp caught from Saginaw Bay or the Great Lakes than from other locations in this country since it contradicts Stansby's (1973) classification of carp as a generally low fat (under 5%), high protein (15-20%) species. Stansby (1973) noted that summer carp occasionally fall into the medium fat (5-15%),

TABLE 3.--Yields of carp processed through mechanical deboner, n = 8 replications.

Whole(kg)	Dressed(kg)	Yield(%±s.e.)	Mince(kg)	Yield(%±s.e.)[1]	Net Yield(%±s.e.)[2]
512.6	310.7	61±1.1	213.5	69±1.8	42±1.2

[1]Percent of dressed weight.

[2]Percent of whole fish weight.

TABLE 4.--Characteristics of mechanically deboned carp harvested during winter and spring.

Month	Composition(%)			Bone & scale (%)	Color			Shear force (lb/g)	TBA (mg MA/1000g)	Total plate count (no./g)
	Moisture	Fat	Protein		L	a	b			
Dec.	68.43	15.46	13.42	0.034	40.9	10.1	11.3	0.305	2.19	$1.5x10^4$
Dec.	68.48	17.06	13.54	0.047	40.8	8.9	11.6	0.262	1.26	$1.1x10^5$
Jan.	72.09	12.85	12.94	0.026	37.6	15.0	11.7	0.334	0.71	$2.5x10^4$
Feb.	72.35	14.00	13.30	0.055	37.0	14.0	11.4	0.377	0.66	$1.9x10^4$
Apr.	61.57	24.57	13.03	---	35.4	14.1	10.6	---[1]	1.49	$3.0x10^4$
May	68.55	15.94	14.12	---	33.1	15.1	11.4	---[1]	1.31	$6.6x10^5$
mean	68.58	16.65	13.39	0.040	37.5	12.9	11.3	0.309	1.27	$1.4x10^5$
±s.d.	±3.89	±4.15	±0.42	±0.013	±3.0	±2.7	±0.4	±0.048	±0.56	$\pm 2.6x10^5$

[1]Shear press malfunction.

high protein (15-20%) category. Although proximate composition was not determined for intact muscle it is doubtful that the mechanical deboning process was primarily responsible for the discrepancy. Results of Angel and Baker (1977) for Israel pond raised carp are more similar to the findings of this study. They determined an average water, fat and protein content of 73.1%, 9.2% and 16.7% respectively in fillets and of 58.2%, 26.1% and 12.9% respectively in frames (fillet waste). Although not discussed by these researchers much of the lipid material associated with the frames was probably adipose tissue deposited on the caecal wall and in the muscle dorsal to the backbone. This material would be expected to elevate fat content and depress moisture and protein percentage in the minced flesh following mechanical deboning.

Proximate composition varied appreciably among batches of carp notably in fat content with a concomitant change in moisture content (Table 4). Batches are listed by month of catch but in addition to seasonal shifts, variability may be attributed to different sex ratios or average weights due to changing catch methods or fishing locations.

Bone and scale content (0.04%) averaged only slightly higher than in the preliminary study. Nearly all residue consisted of bone fragments estimated at 4mm or smaller in length. These results compare favorably with a survey conducted by Patashnik et al. (1974). In that study commercially prepared minced cod blocks from Canada contained 1.2 bones/100g with none longer than 6.3mm while similar blocks imported from Denmark contained 102 bones/100g,

9% of which were longer than 6.3mm. U.S. standards for minced fish blocks permit up to 108 hard bones longer than ¼ inch (6.3mm) per 5 pounds to qualify for grade A status (Federal Reg., 1979_a).

Considerable variation in color was recorded during the course of the study (Table 4). Although not separately quantified, males were visibly darker and redder than females and large fat females (∿7.5kg) appeared more yellow than smaller, leaner females. These observations are partly supported by the occurrence of a significant positive correlation ($r=+.902$, $p < .05$) between fat content and Hunter "+b" value, a measure of yellow pigmentation (Table 5). However, no significant correlations existed between fat or moisture content and color whiteness or redness. The two December batches consisted entirely of females and were lightest in color and the least red.

The Hunter color values for minced carp listed in Table 4 describe a more highly pigmented species than most reported in the literature. Baker et al. (1977) measured three minced gadoid species (e.g. cod) that averaged L, a and b values of approximately 58, -1 and +8, respectively. Mullet is known to contain more red muscle than gadids and was measured at respective L, a and b values of near 50, +3 and +8 (Baker et al., 1977). The prevalence of heme pigments in certain marine species is usually attributed to intensive physical activity which requires aerobic metabolism. In carp the large concentration of hemeproteins is probably better explained by the species adaptability to warm, low dissolved oxygen environments.

The relatively more yellow color of carp (larger "b") compared to the other species mentioned above may be related to carotene content although this was not validated. Raw carp fillets possess an average vitamin A activity of 170 I.U./100g while cod fillets contain no vitamin A activity (USDA, 1963). Carotenes are fat soluble pigments and when present in the diet may accumulate more readily in fatty fish. The possibility that yellow pigments were byproducts of lipid oxidation is unlikely since a weakly negative correlation coefficient (-.40) existed between Hunter "b" and TBA values, Table 5.

The shear force values in Table 4 are not readily compared to the literature since most studies sheared only cooked samples. Resistance to shear force appeared to positively correlate with water content ($r = +.87$). The result seems contrary to the findings of Love (1975) who reported a slightly softer texture (sensory evaluated) in cooked cod with high water content. One explanation may be that of greater cellular turgidity in the raw carp muscle with moderately higher moisture content coupled with Love's observation that cod with very high water contents were usually spawned and depleted of glycogen. Such cod did not experience a characteristic drop in pH and were soft and moist when cooked. Also, a negative correlation coefficient between shear force and fat content ($r = -.81$) may indicate a lubricity effect or a hydrophobic disruption of the myofibrillar protein continuum in samples containing considerable quantities of free oil (Table 5).

TABLE 5.--Selected correlation coefficients for parameters of freshly minced carp.

	Moisture	Fat	Bone & scale	Color			Shear force	TBA	Total plate count
				L	a	b			
Moisture	1.0	--	--	-.21	+.07	--	+.87	-.54	--
Fat		1.0	--	--	--	+.90*	-.81	+.37	--
Bone & scale			1.0	--	--	--	-.18	--	--
Color a					1.0	--	--	-.54	--
Color b						1.0	--	-.40	--
Shear force							1.0	--	-.77

*Significant, $p<.05$.

Correlation of texture with total plate count may suggest an inverse trend ($r = -.77$). Uncontrolled variables of fish freshness, processing time and temperature may have resulted in some batches with elevated bacterial counts and concurrent softening due to proteases. Although some protein hydrolysis may be traced to microbial activity, native acid and alkaline proteases are perhaps more important during the early stages of fresh storage (Pedraja, 1970; Lin et al., 1980). Total plate counts varied considerably in freshly minced carp but the mean of 1.4×10^5 organisms/g is consistent with typically reported values. Nickelson et al. (1980) determined a low of 1.2×10^5/g and a high of 2.6×10^8/g for mince made from six Gulf of Mexico species. Counts reported by Raccach and Baker (1978) on finished minced fish products ranged from 4.7×10^5/g go 7.0×10^5/g.

Part II: Antioxidants

TBA number is commonly used as an index of lipid oxidation in foods and is generally thought to result from the condensation of malonaldehyde (a reactive dicarbonyl) with thiobarbituric acid to form a red chromogen. Results of the lipid storage stability study comparing untreated minced carp with mince containing either of two commercial antioxidants are summarized in Table 6 and Figure 7. Thiobarbituric acid (TBA) number generally increased during frozen storage after 6 months for control and Freez-gard treated samples and after 9 months for Tenox 2 treated samples. This apparent increase was confirmed by a significant variance ratio (F statistic for the time factor, $p<.01$). See Appendix A.1 for

TABLE 6.--Effect of antioxidants on TBA number (mg malonaldehyde/1000 g) during frozen storage at -26°C, 3 replications per treatment.

Treatment	Time, mo. 0	1	2	3	6	9	12	Mean
Control	1.74	2.16	1.73	2.04	2.85	4.51	7.81^{W}	3.26
Freez-gard	2.48^{1}	2.36	1.90	2.45	2.90	3.39	5.24^{WX}	2.96
Tenox 2	1.14^{1}	0.60	1.13	1.33	1.18	1.43	2.29^{X}	1.30
Mean	1.79^{ab}	1.71^{ab}	1.58^{a}	1.94^{ab}	2.31^{b}	3.11^{c}	5.12^{d}	

Standard errors of differences of means

Time	0.53
Treatment	0.97
Time x treatment	1.29

^{abcde}Values within rows designated by different superscripts are significantly different ($p<.05$).

^{wx}Values within columns designated by different superscripts are significantly different ($p<.05$).

^{1}The first of three replicates for the month 0 mean TBA value was missing and was estimated by iterative least squares analysis.

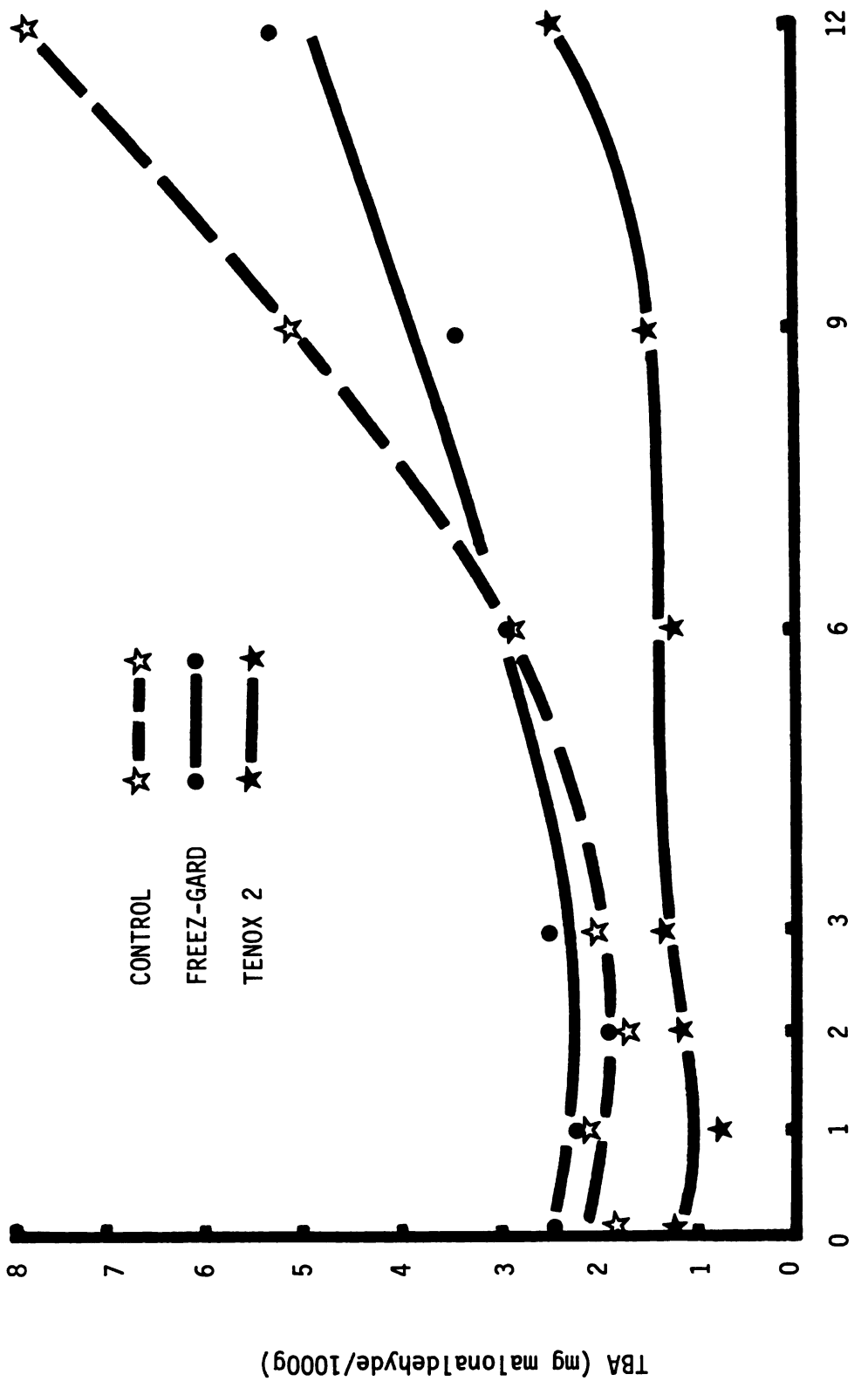

Figure 7. Effect of two antioxidants on TBA values of minced carp during frozen storage at -26°C.

analysis of variance. Combining all treatments and analyzing means produced significant differences ($p<.05$) between 9 month and 12 month TBA values and between these values and all preceding times (Table 6).

Similar trends were reported by Morris and Dawson (1979) for mechanically deboned sucker. Although an increase in TBA number is expected during storage some investigators have described a decline after an initial peak (Botta and Richards, 1973; Wood et al., 1971). Declining TBA values indicate a loss, degradation or immobilization of malonaldehyde, its precursors, or of other TBA reactive derivatives. Kwon et al. (1965) showed that TBA reactive substances react with protein and become partially unavailable for measurement by TBA analysis. Buttkus (1967) reported the binding of malonaldehyde to amino groups of trout myosin and noted that this occurred at a faster rate at -20°C than at 0°C.

The effective removal of malonaldehyde or parent compounds by binding to protein may explain the slight decline in TBA number at month 1 in Tenox 2 treated mince and at month 2 in the control. The protein-bound malonaldehyde theory suggests a dynamic system where TBA number is a function of relative rates of malonaldehyde production and protein interaction. The early drop in TBA values below initial levels resulted in a significantly lower mean TBA value at month 2 than at month 6 ($p<.05$). This was the only significant difference during the first 6 months of storage (Table 6). In mechanically deboned carp the TBA method appears most valid as an index of lipid oxidation after 3 to 6 months of storage.

Despite the lower TBA numbers determined for the Tenox 2 treatment compared to the control or Freez-gard treatment, the treatments were not significantly different over the course of the study (Appendix A.1). Perhaps due to differences in composition or handling among batches of fish, the F statistic proved highly significant for replication variance (p much less than .001). To determine if this variation was consistent (e.g. the first TBA replication higher than the other two for the control treatments and times) analysis of variance was also conducted on difference values. That is, prior to statistical examination, TBA values for each treatment and time were subtracted from the corresponding control TBA value, as suggested by Snedecor and Cochran (1967). This reduced the replication variance but it was still significant ($p<.05$, Appendix A.2). Making a logarithmic transformation of the data (TBA+1) before subtracting treatment from control values produced no additional information.

Treatment x time interaction was characterized by a weakly significant F statistic ($p<.1$, Appendix A.1), however analysis of means was deemed warranted. Only one difference was found. The Tenox 2 treatment had a significantly smaller mean TBA number than did the control at the 12 month sampling date ($p<.01$, Table 6). Apparently Tenox 2 is more effective than Freez-gard as an antioxidant in mechanically deboned carp but its benefits are most realized after extended storage.

These results are dissimilar to a study on minced sucker by Morris and Dawson (1979) where Freez-gard (a mixture of sodium

hexametaphosphate, NaCl and sodium erythorbate) was found to be far superior to the control and three phenolic antioxidants (including Tenox 2) in reducing TBA values. The authors noted that phosphates are capable of sequentering metals and holding water and that erythorbate can interfere with heme catalyzed oxidation (Watts, 1950). The synthetic antioxidants were thought to not distribute evenly throughout the flesh due to their poor solubility. Iredale and York (1977) also studied minced sucker and reported antioxidant activity for erythorbate but not for tripolyphosphate or ethylenediamine tetracetic acid (EDTA). It would seem that free metal catalyzed oxidation in minced sucker is less important than heme catalyzed oxidation or other mechanisms. Reducing agents such as erythorbate are readily oxidized and probably function as oxygen scavengers, thereby protecting pigments and lipids (Labuza, 1971).

The minced carp used in this study is much higher in lipid content and probably heme pigment content than sucker or many other species. The natural oil may have served as an effective carrier for Tenox 2, consequently overcoming the problem of distribution. Also, Freez-gard was added at the same level as in the study by Morris and Dawson (1979) which corresponds to a smaller antioxidant:fat ratio in minced carp.

Tenox 2 is primarily a type 1 antioxidant, containing the synthetic compounds butylated hydroxy anisole (BHA, 67%) and propyl gallate (PG, 20%) but also citric acid (13%). BHA and PG act as free radical chain terminators by donating a hydrogen without the phenolic residue entering into further propagation reactions:

$$ROO\cdot + AH \longrightarrow ROOH + A\cdot$$
$$R\cdot + AH \longrightarrow RH + A\cdot$$
$$A\cdot + X \longrightarrow AX$$

where AH represents the antioxidant, ROO· are peroxy radicals and X is some other moeity. The goal is to inactivate enough radicals to appreciably delay the onset of exponential degradation reactions.

Yu et al. (1969) reported a significant reduction in TBA and peroxide values during frozen storage of salmon steaks when treated with BHA and BHT in a starch glaze to improve antioxidant contact with the fish. When mixed with minced black rockfish, BHA and BHT extended shelf life approximately 4 fold.

Due to the relative ineffectiveness of Freez-gard and the very low concentration of citric acid contributed by Tenox 2 (.002% by weight of mince), radical chain termination was probably more important than trace metal chelation in the mechanism by which Tenox 2 protected lipids. Moledina et al. (1977) chelated metals in mechanically deboned flounder with citric acid applied at the rate of 0.3 to 0.5%.

The NaCl in Freez-gard is added to control texture but may have demonstrated prooxidant activity. Sodium chloride and its metallic impurities (e.g. Cu, Fe, Cr) are known to catalyze lipid oxidation in some red meat products (Rust and Olson, 1973). A number of researchers have determined that ascorbic acid can act as a prooxidant as well as an antioxidant depending on concentration, substrate and environmental factors (Deng et al., 1978; Moledina et al., 1977). Perhaps at the level used (∿100ppm) in minced carp the oxygen consumption ability of erythorbate (a salt of the D-isomer

of ascorbic acid) was largely neutralized by its simultaneous activation of heme or nonheme iron catalyzed oxidation. Nonheme iron may be significant even in a chelated system since very low concentrations of certain metallic ions are often sufficient for initiation.

Excess heme and heme proteins may actually inhibit oxidation possibly by formation of porphyrin derivatives capable of attacking free radicals (Hirano and Olcott, 1971). This factor complicates the expected role of hemoglobin and myoglobin as catalysts in minced carp. Carp lipids should be highly vulnerable to oxidative rancidity due to the presence of many double bonds. In a study of cooked minced carp, Mai and Kinsella (1979) reported a fatty acid profile consisting of more than 75% unsaturated fatty acids including 33% polyunsaturated fatty acids of which 35% contained 5 or 6 double bonds.

The TBA numbers recorded in Table 6 and Figure 7 are nearly twice the magnitude of those to be discussed in the next section, "Corrective Treatments." Since the procedure required mixing of the control and antioxidant treated samples it is likely that oxidation was encouraged by incorporation of oxygen and by increasing the contact of reactants with substrate.

The TBA values from this study will not be directly compared with the results from other laboratories because of susceptability of the test to differences in procedure. However, the study by Morris and Dawson (1979) was performed on minced sucker under essentially identical conditions and demonstrates some important

concepts. The control TBA values rose from 0.3 at day 0 to 3.6-9.0 at 12 months, depending on season of harvest. Despite much higher lipid and pigment content in carp than sucker, mean TBA values at 6 months were similar (2.85 vs 2.7, respectively). The large seasonal variability experienced in the sucker study was apparently caused more by compositional changes in the lipid (e.g. percentage of polyenes or phospholipids) or in catalyst concentration than by quantity of lipid which ranged seasonally from 2.8% to 5.4%. Those findings illustrate the need for future investigation of seasonal impact on carp storage stability, a variable not considered in the current study.

No sensory evaluation of the minced carp was conducted during storage which makes a prediction of expected storage life very difficult. In coho salmon steaks treated with antioxidants, Yu et al. (1969) related unacceptably rancid flavor with a TBA value as low as 0.8 using the TBA method of Yu and Sinnhuber (1967). This is well below values determined for month 0 Freez-gard treated and untreated minced carp. The distillation TBA procedure (Tarladgis et al., 1960) may synthesize TBA reactive compounds; a condition suggested by the smaller initial TBA numbers in samples containing Tenox 2.

Part III: Corrective Treatments

Based on the premise that mechanically deboned carp from Saginaw Bay is too highly pigmented and flavored for maximum utilization in its native state, the corrective measures described in the "Experimental" section were analyzed with the following results.

Heme Pigments

All washed treatments and the H_2O_2 treated mince were significantly lower in hemeprotein content than the control ($p<.05$, Table 7). Of these, the water washed mince with 5% hydrogenated vegetable fat added (= plus-fat treatment), the mince washed with $NaHCO_3$ solution (=$NaHCO_3$ treatment) and the H_2O_2 treated mince contained less hemeprotein than the control at the 5% level of significance. The hexane extracted mince to which 5% hydrogenated vegetable fat was added (= hexane treatment) was not different than the control, water wash or NaCl wash treatment but was higher in hemeprotein content than the plus-fat, $NaHCO_3$ and H_2O_2 treatments ($p<.05$).

The elution of heme pigments by washing in water or weak ionic solutions was expected due to the soluble nature of these proteins. An unexpected result was the very large reduction in measured hemeprotein of the plus-fat treatment. Although intended in part to dilute heme content or to whiten apparent color (Lanier and Thomas, 1978), dilution by the addition of 5% fat can only account for 5.5% of the observed decrease. The procedure for heme pigment determination produced similar slightly turbid supernatants for spectrophotometric analysis in all samples except the plus-fat samples which appeared clear. Problems with turbidity were also reported by Rickansrud and Henrickson (1967). Possibly the unsaturated fish oils aggregated by hydrophobic interaction with the harder hydrogenated fat during blending and were then more readily removed by cold centrifugation.

No additional benefit resulted from the use of NaCl in the primary wash. The determined hemeprotein concentration was essentially the same as the plain water washed treatment (Table 7). Sodium Chloride applied as a weak solution (0.5%) was expected to expedite the removal of water soluble pigments by partially unfolding their tertiary structure without measurably solublizing myofibrillar proteins. On the basic side of a protein's isoelectric point a predominance of negatively charged groups will commonly experience repulsion from otherwise positively charged groups in the presence of NaCl or other salt. This is because Cl^- ions form salt bridges with positive charges and Na+ ions bind to negative charges producing adjacent groups that carry electrostatically similar charges (Cann and Phelps, 1955). The condition should increase solubility by exposing additional sites suitable for hydrogen bonding with water. Very little myofibrillar protein was expected to solubilize since cross-linkages inhibit their extraction in solutions up to 2.5% NaCl (Brotsky and Everson, 1973).

In direct contrast to anticipated results, small aggregates of red pigment were noticed in at least one of the NaCl treated replicates. Possibly this was due to an effect discussed by Dawood (1979). Adding a small quantity of NaCl to a negatively charged protein may cause preferential absorbtion of Na^+ ions and thereby reduce the charge to the isoelectric point (Mahon, 1961). Residual NaCl in the dewatered mince was 0.099% attributable to the treatment (Table 8).

TABLE 7.--Total heme pigment and pH of treated minced carp, 2 replications per treatment.

Treatment	Heme pigments (mg/g)	pH
Control	3.99^{a}	6.70^{ab}
Water wash	1.87^{bc}	6.92^{a}
Water wash plus fat	0.18^{b}	6.98^{a}
NaCl wash	1.95^{bc}	6.88^{a}
$NaHCO_3$ wash	0.45^{b}	7.76^{ac}
H_2O_2	0.74^{b}	6.00^{1}
Hexane extracted	2.81^{ac}	6.71^{ab}

[1]Final adjusted pH.

[abc]Means within columns designated by different superscripts are significantly different ($p<.05$).

TABLE 8.--Residual sodium chloride in sodium chloride washed minced carp, 3 replications per treatment.

Treatment	Chloride as NaCl (%)
Control	0.080^{a}
NaCl wash	0.179^{b}
Difference	0.099

[ab]Means designated by different superscripts are significantly different ($p<.05$).

The $NaHCO_3$ treated flesh was found to be lower in heme pigment content than the water wash or NaCl treatment, although the difference was not significant (Table 7). On a weight basis $NaHCO_3$ should afford less ionic strength and donate fewer ions than NaCl. However, if differences in hemeprotein concentration exist, they may be best explained by pH. A mean pH of 7.76 for the $NaHCO_3$ treatment was the only pH significantly higher than the control or hexane treatment ($p<.05$, Table 7). The H_2O_2 treatment had been chemically adjusted to pH 6.00 and was not included in the statistical analysis.

As previously discussed, proteins in an environment that is on the alkaline side of their isoelectric point carry a net negative charge. Their conformation becomes more open and their ability to interact with water is increased. The effect appears to be especially pronounced in hemoglobin which contains no covalent cross-linkages and is conformationally sensitive to small changes in H^+ concentration as part of its physiological function (Fairley, 1980). At the elevated pH encountered by the use of $NaHCO_3$, unfolding of the peptide chains may have increased hemoglobin and, to a lesser extent, myoglobin solubility.

The H_2O_2 treatment decreased hemeprotein concentration significantly compared to the control and hexane treatment ($p<.05$) but did not provide the near total porphyrin destruction expected (Table 7). Although hydrogen peroxide is a strong oxidizing agent the treatment schedule followed was developed for mechanically deboned cod frames which may not be as highly pigmented as minced carp (James and McCrudden, 1976).

The hexane extracted mince was not intended to reduce heme pigment content, however a very small amount was visibly removed with the solvent (Table 7).

Proximate Composition

Variation among treatments in moisture, fat and protein percentages was significant ($p<.01$, Appendix A.3). Moisture content was significantly elevated above the control or hexane treatment levels for all wash treatments except plus-fat ($p<.05$, Tables 9 and 10). Since the washing procedure dictated removal of fat, compensation in the water and protein fractions on a percentage basis was predicted. However, measured protein content also decreased nearly 30% in all wash treatments. Dewatering was not assisted by centrifugation or pressure and consequently some added moisture was evidently retained. Patashnik (1974) reported a 9.6% increase of water in washed minced carp compared to a 15.5% increase found in this study. Patashnik's method of dewatering was not described but in a later study the same laboratory controlled water concentration with a Bock centrifuge (Patashnik, 1976). The H_2O_2 and hexane treatments did not significantly alter moisture content.

The washes and hexane treatment significantly reduced percentage fat ($p<.05$) to 35 to 56% of their corresponding control (Table 9). With a final mean composition of 8.32% fat, hexane extraction was responsible for a reduction from an initial 16% to 3% fat prior to the addition of 5% vegetable fat.

The apparent loss of protein following all wash treatments was confirmed by an appreciable concentration of protein determined

TABLE 9.--Effect of treatments on parameters of minced carp, expressed as mean percent of corresponding control.

	Composition			Color					
Treatment	Moist.	Fat	Prot.	L	a	b	Total plate count	TBA	Shear force
Controls	100^{w}	100^{w}	100^{xy}	100^{2}	100^{wy}	100^{wy}	100^{w}	100^{w}	100^{wy}
Water wash	123.0^{x}	40.2^{x}	71.8^{wy}	140.0^{w}	54.4^{w}	111.1^{w}	43.6^{w}	85.6^{w}	165.1^{wxyz}
NaCl Wash	123.5^{x}	38.2^{x}	70.4^{w}	142.7^{w}	54.3^{w}	109.4^{w}	31.4^{w}	127.4^{w}	187.0^{xz}
$NaHCO_3$ wash	124.3^{x}	34.9^{x}	72.8^{wy}	143.2^{w}	51.9^{wz}	110.6^{w}	32.6^{w}	73.7^{w}	178.7^{yz}
Water wash plus fat	120.2^{wx}	56.1^{x}	71.6^{w}	139.7^{w}	56.0^{w}	106.4^{w}	--	88.8^{w}	86.0^{w}
H_2O_2	100.2^{w}	88.7^{w}	94.9^{wxy}	134.2^{w}	-10.0^{x}	86.8^{x}	16.2^{w}	168.8^{w}	94.2^{w}
Hexane extracted	107.9^{wx}	50.9^{x}	119.2^{x}	109.0^{w}	64.0^{xy}	92.8^{xy}	291.0^{w}	133.9^{w}	110.2^{wxyz}

^{wxyz}Means within columns designated by different superscripts are significantly different ($p<.05$).

TABLE 10.--Effect of treatments on properties of minced carp.[1]

Treatment	Composition			Color			Total plate count (log No./g)	TBA (mg MA/1000g)	Shear Force (lb./g)
	Moist.	Fat	Prot.	L	a	b			
Control (washes) n=4	68.6 (5.0)	17.1 (5.3)	13.2 (0.3)	37.7 (2.3)	13.0 (2.8)	11.3 (0.5)	4.7 (0.3)	1.03 (0.4)	0.32 (0.1)
Water wash n=4	84.1 (2.1)	6.5 (1.6)	9.5 (1.5)	52.6 (1.6)	6.9 (0.9)	12.6 (0.5)	4.3 (0.4)	0.81 (0.1)	0.53 (0.1)
NaCl wash n=3	84.3 (0.7)	5.9 (1.1)	9.2 (2.4)	52.3 (3.7)	7.9 (3.0)	12.3 (0.2)	3.9 (0.2)	1.01 (0.2)	0.66 (0.0)
$NaHCO_3$ wash n=3	85.0 (1.3)	5.6 (1.2)	9.5 (0.8)	52.5 (3.1)	7.5 (2.7)	12.4 (0.1)	3.9 (0.1)	0.64 (0.1)	0.63 (0.1)
Control (wash+fat) n=2	67.0 (7.6)	19.3 (7.5)	13.2 (0.2)	45.6 (14.4)	9.9 (6.4)	11.8 (1.7)	--	1.08 (0.6)	0.60 (0.0)
Water wash plus fat n=2	80.0 (1.4)	10.0 (0.0)	9.4 (1.3)	60.7 (1.3)	4.6 (0.8)	12.5 (1.0)	--	0.85 (0.1)	0.51 (0.0)
Control (H_2O_2) n=2	69.0 (0.7)	16.7 (1.0)	13.2 (0.3)	49.1 (6.4)	12.6 (1.9)	14.3 (0.2)	4.7 (1.1)	1.30 (0.7)	0.36 (0.6)
H_2O_2 n=2	69.2 (1.8)	14.8 (2.1)	12.5 (0.2)	65.5 (3.2)	-1.2 (0.1)	12.4 (1.3)	2.9 (0.1)	1.97 (0.3)	0.34 (0.0)
Control (hexane extracted) n=2	68.8 (0.4)	16.3 (0.6)	13.9 (0.4)	36.4 (4.7)	14.4 (1.0)	11.7 (0.4)	5.6 (0.5)	1.08 (0.3)	0.12 (0.0)
Hexane extracted n=2	74.2 (0.1)	8.3 (0.2)	16.5 (0.6)	39.4 (1.8)	9.2 (1.6)	10.8 (0.1)	5.8 (0.1)	1.69 (0.7)	0.13 (0.0)

[1] Treatment mean (standard error)

in the wash solutions (Table 11). Protein concentrations in solutions of the three wash treatments were not different although NaCl and $NaHCO_3$ appeared to promote protein loss.

Color

Overall color intensity (Hunter "L") was not significantly affected by treatment (Table 9) although absolute values suggest some whitening resulted from washing (Table 10). No additional improvement in whiteness was indicated by use of NaCl, $NaHCO_3$ or added fat. It was previously reported that $NaHCO_3$ may have expedited the solublization of heme pigments by the influence of pH on conformation. However the higher pH may have been responsible for a condition similar to that reported in beef of abnormally high pH, known as dark cutting beef (Briskey and Kauffman, 1971). The $NaHCO_3$ wash was the only wash treatment that yielded significantly lower Hunter "a" values (less redness) than the control; a finding that usually correlates well with hemeprotein content (Kropf, 1980).

The H_2O_2 treatment demonstrated no more whitening ability than the wash treatments, however it resulted in a very large reduction in Hunter "a" value that was significantly below the control or any other treatment ($p<.05$). This result seems to conflict with hemeprotein loss which was not shown to be greater than for the wash treatments (Table 7). The Hunter "a" value designates a transition scale from green (-a) to red (+a) so that a value of 0.0 can represent a balance of green and red pigments as well as the absence of pigment. Certain H_2O_2 producing bacteria

TABLE 11.--Solublized protein (by Biuret) in wash water of 3 treatments, 3 replications per treatment.

Treatment	protein (mg/ml)
Water wash	8.55[a]
NaCl wash	9.28[a]
$NaHCO_3$ wash	9.44[a]

[a]Treatments designated by different superscripts are significantly different ($p<.05$).

are known to cause the oxidation of heme to a green pigment (Giffee et al., 1960), which supports the finding of negative "a" values following H_2O_2 treatment.

The hexane treatment resulted in some reduction in redness but had less effect on Hunter "L" and "a" values than the other treatments (Table 9). A moderate decline in the Hunter "+b" value was sufficient to describe the reduction of yellow pigmentation due to the hexane treatment as different from the plain water, plus-fat, NaCl and $NaHCO_3$ treatments ($p<.05$) in which relative yellowness increased slightly. Hexane was more effective than the washes at removal of lipids, a fraction that includes carotenes.

A slightly more pronounced decrease in yellow coloration was evident following H_2O_2 treatment, the only treatment significantly different than the control ($p<.05$, Table 9). The carotenoid component may be particularly vulnerable to oxidation by H_2O_2. In a study by Sims et al. (1975) marinated herring (salt-acetic acid cured) soaked in 600ppm H_2O_2 were off-white with an apparent "yellow cast." The inconsistency between their findings and those reported here might be attributable to a pH effect in their study that favored the formation of yellow free and oxidized hemes or to H_2O_2-stable yellow compounds derived from pretreatment enzymatic or nonenzymatic reactions.

Texture

The NaCl treatment was the only treatment significantly different from the control for its resistance to shear ($p<.05$, Table 9). Many authors have reported the use of NaCl to firm texture

in comminuted fishery products (Bello and Pigott, 1979; Miyauchi et al., 1975; Iwata and Okada, 1971) and is usually credited with partial solublization of proteins (principally myosin) which form an intimate interactive network serving as a "cement" between particles (Lee and Toledo, 1976; Patashnik et al., 1976). However minced carp was not chopped with NaCl and this effect seems unlikely at the low level of NaCl remaining after washing.

The plain water wash and $NaHCO_3$ wash also appeared to increase shear resistance slightly. This was expected due to (1) removal of water soluble proteins which have less effect on binding than myofibrillar proteins, (2) improvement of the myofibrillar protein continuum and (3) elution of softening enzymes (Okada et al., 1973).

The plus-fat treatment reduced shear resistance compared to the NaCl and $NaHCO_3$ treatments ($p<.05$), probably due to protein dilution and lubricity. The extra firmness displayed by $NaHCO_3$ treated mince was unexpected since fish of unusually high pH (>7.0) are generally characterized by a soft or "sloppy" texture (Love, 1975; Kelly et al., 1966). The soft condition reported in these cooked fillets is often accompanied by increased hydration. an increase in bound water (not necessarily total water) might serve to strengthen raw texture by swelling and yet inhibit formation of crosslinks between proteins when cooked. Evidence will be presented in a later section, "Effect of Cooking on Treatments" to support this explanation for $NaHCO_3$ treated minced carp. No apparent texture change resulted from H_2O_2 or hexane treatment (Table 9).

Effect on Microbial Status and Lipid Oxidation

None of the treatments were significantly different from each other or their corresponding control for total plate count or TBA value (Table 9). Lee and Toledo (1977) also found no significant reduction in TBA value by washing minced fish if it was then stored fresh. However, the plain water, plus fat and $NaHCO_3$ washes appeared to reduce TBA number slightly (Table 10), possibly due to loss of polyunsaturated lipids and removal of heme or metallic catalysts. By contrast, a slight prooxidant effect was introduced by NaCl treatment. Increased TBA numbers associated with H_2O_2 and hexane treatments may reflect the elevated temperatures encountered during processing or other factors that are discussed in a later section.

Bacteria counts dropped after exposure to H_2O_2 which is sometimes used medicinally as a topical antiseptic. James and McCrudden (1976) believed antimicrobial activity to be an important benefit of high pH H_2O_2 bleaching of minced cod frames. Whether the 84% reduction in bacterial load observed in minced carp translates to extended shelf life is not clear without further study (Table 9).

Long exposure to warm conditions was probably responsible for an apparent increase of organisms following hexane treatment. Lower plate counts after washing was probably due to removal of bacteria with the wash water.

Bone and Scale

The possibility that removal of oil and water soluble components during the wash procedure might concentrate bone and scale residue was investigated. Although an increase was noted, it was not significantly different than the control (Table 12).

Lipid Stability During Frozen Storage

The effect of all treatments on production of TBA reactive compounds during storage is summarized by difference from control in Table 13 and illustrated by actual TBA numbers in Figures 8-11. Analysis of variance for difference values produced F statistics that were significant for effect of time and treatment on TBA numbers (Appendix A.4). TBA values for months 9 and 12 were significantly higher than previously determined times ($p<.05$) and month 12 had higher TBA numbers than month 6 ($p<.05$, Table 13). Figures 8-11 suggest that the controls and hexane treatment were primarily responsible for the significant lipid oxidation after 6 months. Data for the 4 wash treatments are plotted on two figures (8 and 9) for clarity, however the control is the same and they are directly comparable.

Analysis of treatment mean differences by Tukey's or Student-Neuman-Keul's procedure (Steel and Torrie, 1960) yielded no significant distinctions among treatments. However, when treatments were compared only to their control by Dunnett's procedure (Steel and Torrie, 1960) the $NaHCO_3$ mean TBA number proved significantly lower

TABLE 12.--Bone and scale content as affected by water wash treatment, n=3 replicates.

Treatment	Bone and Scale (%)
Control	0.043[a]
Water wash	0.051[a]

[a]Means designated by different superscripts are significantly different ($p<.05$).

TABLE 13.--Effect of treatments on lipid stability during storage at -26°C, recorded as TBA difference values (TBA number of control minus TBA number of treatment).

	Time, mo.							
	0	1	2	3	6	9	12	Mean
Control[1]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{X}
Water wash[1]	0.14	0.04	0.39	0.47	1.13	1.90	2.48	0.93^{XW}
NaCl wash[1]	-0.05	0.02	0.33	0.27	0.96	1.76	2.80	0.87^{XW}
$NaHCO_3$ wash[1]	0.32	0.58	0.65	0.75	1.40	2.08	2.85	1.24^{W}
Water wash plus fat[2]	0.14	0.28	0.37	0.29	1.10	1.95	2.90	1.00^{XW}
H_2O_2[2]	-0.75	-0.96	-1.17	-0.66	-0.19	1.48	2.82	0.08^{XW}
Hexane extracted	-0.69	-0.26	-0.29	-0.39	0.76	1.57	2.00	0.39^{XW}
mean	-0.13^{a}	-0.04^{a}	0.04^{a}	0.10^{a}	0.74^{ab}	1.53^{bc}	2.26^{c}	

Standard errors of differences of means

Treatment = 0.255

Time = 0.290

Time x treatment = 0.689

^{abc}Means within rows designated by different superscripts are significantly different ($p<.05$).

^{wx}Means within columns designated by different superscripts are significantly different ($p<.05$).

[1]Replicated 3 times.

[2]Replicated 2 times.

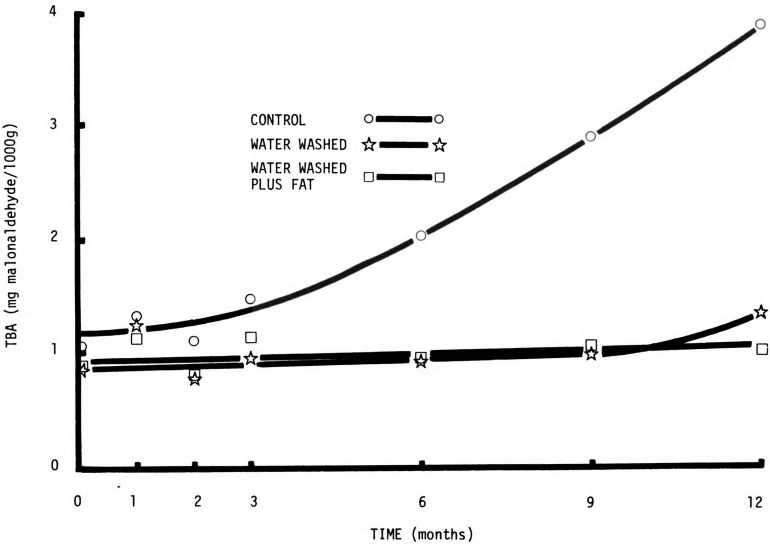

Figure 8. TBA values during frozen storage at -26°C of minced carp treated by water washing or water washing plus 5% added hydrogenated vegetable fat.

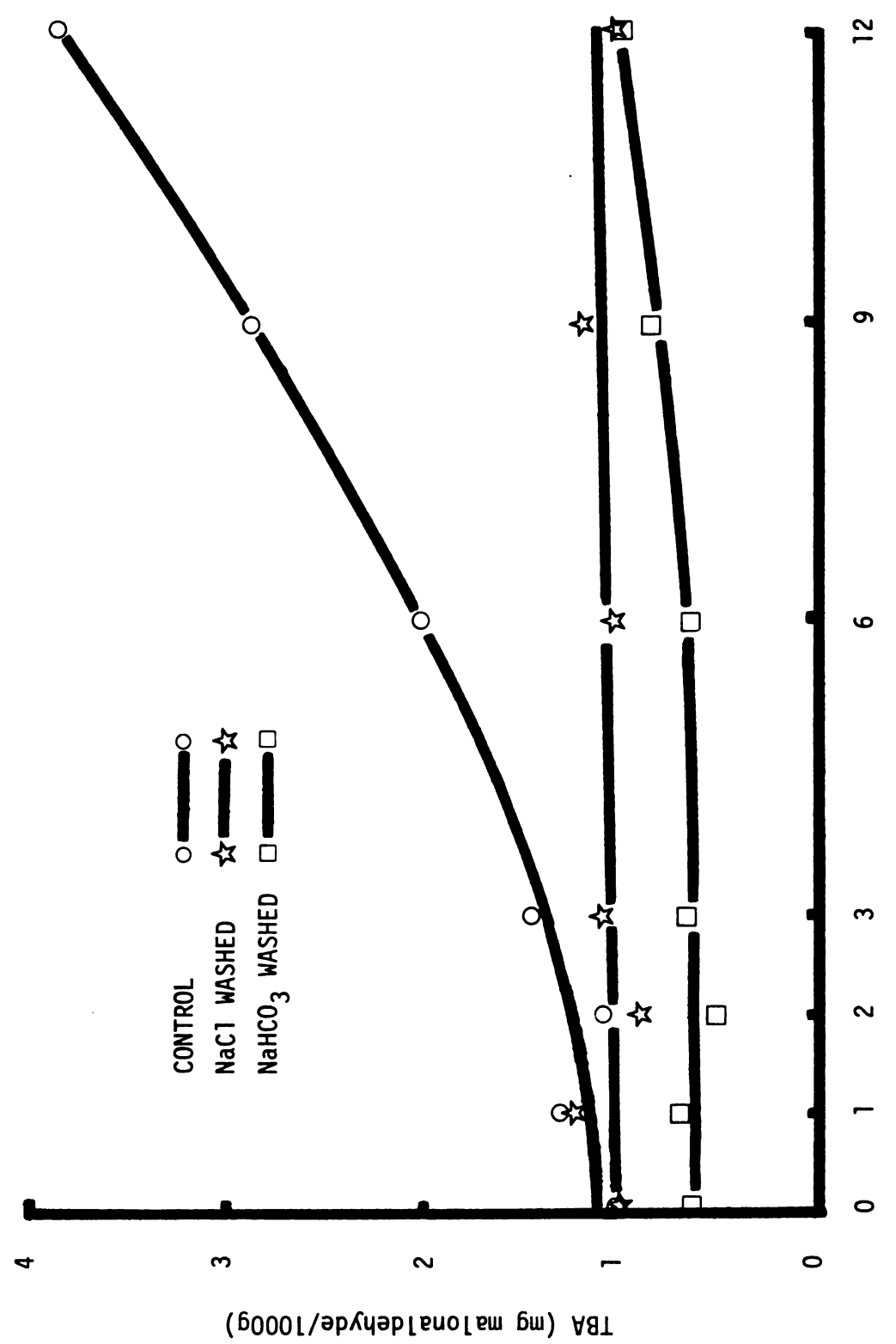

Figure 9. Effect of washing minced carp in 0.5% w/v solutions of NaCl or $NaHCO_3$ on TBA values during frozen storage at $-26^{o}C$.

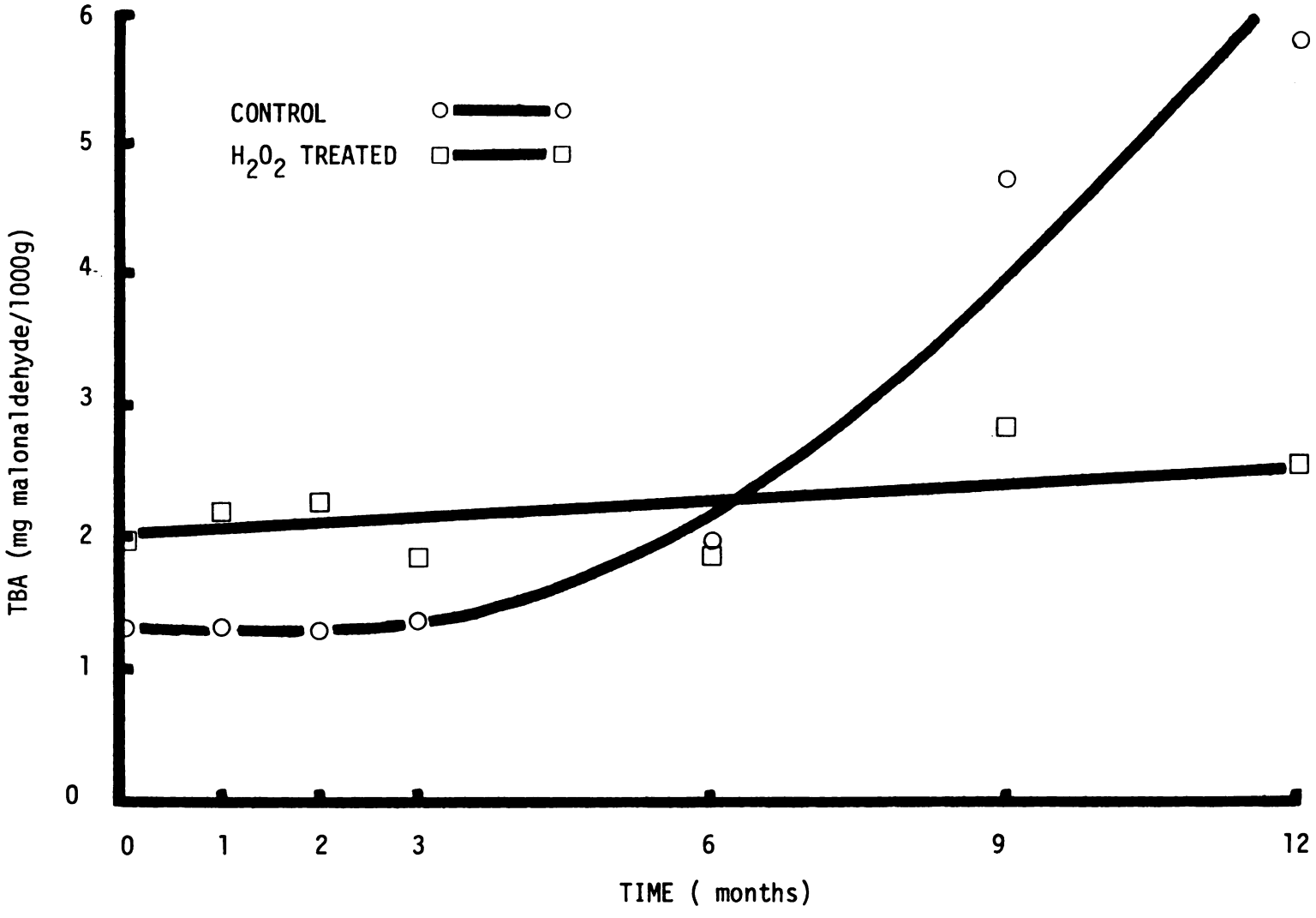

Figure 10. Effect of hydrogen peroxide treatment on TBA values of minced carp during frozen storage at -26°C.

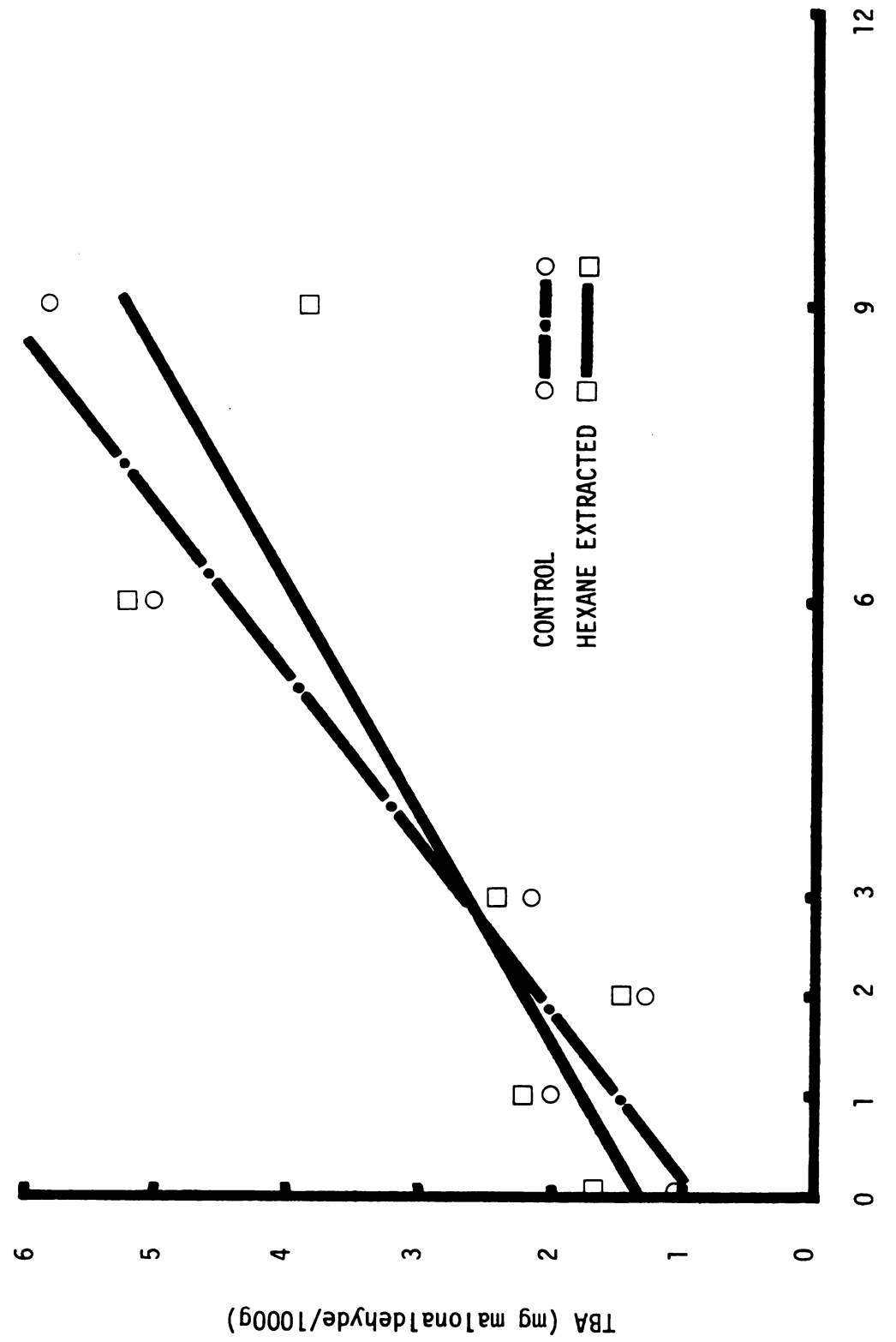

Figure 11. Effect of hexane extraction plus 5% added hydrogenated vegetable fat on TBA values of minced carp during frozen storage at -26°C.

than the control during storage (Table 13). Treatment x time interaction was not significant (Appendix A.4).

The possible inhibition of lipid oxidation by washing can be attributed to reduced lipid content and smaller concentrations of heme and perhaps metallic prooxidants, however the additional protection afforded by $NaHCO_3$ washing is difficult to explain. Perhaps more efficient removal of hemeproteins discussed previously is sufficient to describe the improved stability. Also, pH is known to be related to activation and inhibition of heme and nonheme iron catalyzed oxidation. Wills (1965) determined that nonheme iron is only catalytic at acidic pH with an optimal pH of 5.5. Nonheme iron may be of enzymatic origin as in zanthine oxidase or of non-enzymatic origin as in ferritin. Consequently, the alkaline nature of the $NaHCO_3$ treatment may eliminate nonheme iron as a prooxidant. The explanation may not be that simple and is complicated by evidence that hemeprotein catalysis is accelerated at alkaline pH (Fischer and Deng, 1977).

Use of NaCl or hydrogenated vegetable fat had no appreciable effect on TBA number compared to the plain water fish (Table 13). All of the wash treatments appeared to stabilize lipids and results indicate that washed mince carp resists TBA measured lipid oxidation for at least one year when properly packaged and stored.

TBA values during the first 6 months of storage of the H_2O_2 treated mince were higher than for the washes compared to the controls (i.e. negative differences) (Table 13). A trend similar to the washes was observed in that little change occurred with

time which indicates inhibition of exponential propagation reactions. Several factors may have been involved.

Yu and Sinnhuber (1964) observed a reduction in TBA value when malonaldehyde preparations were exposed to H_2O_2 and suggested that malonaldehyde might be oxidized to malonic acid. Hydrogen peroxide treatment was demonstrated to reduce the rate of lipid oxidation in chicken probably by oxidative destruction of hemeproteins (Lee et al., 1975). If hemeproteins are major catalysts of lipid oxidation in minced carp as suspected, less change in TBA with time should be observed. Simultaneously, non-heme iron catalyzed oxidation may have been activated by the low pH (pH 6.0) prescribed by the H_2O_2 procedure (Fischer and Deng, 1977). Other reasons why the H_2O_2 treatment might be expected to encourage lipid oxidation include the time of exposure to high temperature (16°C) conditions, use of catalase (a hematin enzyme) to cleave residual H_2O_2, direct oxidation of lipids by H_2O_2 and the production of oxygen by the reaction:

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \tfrac{1}{2}\, O_2$$

Apparently none of these factors or their cumulative effect was sufficient to promote free radical propagation as measured by TBA analysis.

Of all treatments, hexane extraction was least different from the control during 9 months of storage (Figure 11). Hydrogenated vegetable fat has been shown to be more stable than carp oil and the solvent was removed under vacuum which might minimize

initiation of lipid oxidation reactions. However, the samples were held approximately 3 hours at 16°C and some residual hexane remained after treatment. According to Labuza (1971) unsaturated lipids oxidize at an increasing rate in solvents of decreasing polarity due to less competition between substrate and solvent for catalysts.

Effect of Cooking

Treatments were compared for relative effect of cooking on color and shear resistance. Because of residual hexane no attempt was made to cook hexane treated mince. Analysis of variance for Hunter color measurements produced significant variance ratios (F) for form (raw versus cooked), treatment and form x treatment interaction except for Hunter "b" data which showed no form x treatment interaction (Appendices A.5-A.7).

Heat denaturation of hemeproteins and permanent oxidation of heme iron account for the loss of color and graying noticed when meat is cooked (Bodwell and McClain, 1971). Consequently, the large mean increase of Hunter L number measured in the control upon cooking is almost certainly a result of high initial concentration of hemeproteins (Table 14). This effect of cooking was responsible for significantly more whitening in the control than in the treatments ($p<.05$). An implication is that products made from treatments designed to lighten color may not realize as much improvement when cooked as expected based on raw color. However, cooked color was still appreciably darker for the control than for the wash treatments and its "L" value for both forms (combined) was significantly smaller (darker) than the treatments ($p<.05$).

TABLE 14.--Effect of cooking on treated minced carp, previously frozen and stored 10 weeks at -26°C, n=3 replicates.

	Treatment															
	Control		Water wash		Water wash + fat		NaCl wash		$NaHCO_3$ wash		H_2O_2		Hexane extracted[3]			
Parameter	raw, cooked[1]	diff.[2]	raw, cooked	diff.	raw, cooked	diff.	raw, cooked	diff.	raw, cooked	diff.	raw, cooked	diff.	raw, cooked	diff.		
L	45.9, 56.7^{a}	-10.8^{a}	59.3, 65.1^{b}	-5.8^{b}	59.6, 64.3^{b}	-4.7^{be}	59.7, 65.9^{b}	-6.2^{bf}	59.0, 61.0^{c}	-2.0^{c}	59.3, 56.6^{d}	+2.7^{d}	47.3, --	--		
a	10.1, 1.2^{d}	+8.9^{c}	2.2, -1.8^{a}	+4.0^{a}	0.8, -1.6^{c}	+2.4^{e}	1.7, -1.9^{ac}	+3.6^{ab}	1.8, -1.3^{a}	+3.1^{b}	-0.5, 0.9^{a}	-1.4^{d}	7.4, --	--		
b	8.9, 10.8^{a}	-1.9^{a}	11.2, 12.6^{b}	-1.4^{a}	10.9, 12.3^{b}	-1.4^{a}	11.1, 12.8^{b}	-1.7^{a}	11.1, 12.7^{b}	-1.6^{a}	11.7, 14.1^{c}	-2.4^{a}	8.8, --	--		
Shear force (lbs/g)	0.32, 1.07^{b}	-0.75^{a}	0.94, 3.32^{b}	-2.38^{b}	1.03, 3.56^{a}	-2.53^{b}	1.04, 2.37^{c}	-1.33^{c}	0.87, 0.69^{b}	+0.18^{d}	0.66, 5.17^{d}	-4.51^{e}	0.37, --	--		
Cook yield[4] (%)	77.0		46.6		44.5		72.0		70.0		69.9		--			

[1]Superscripts following parameter means correspond to total of raw plus cooked. When designated by different superscripts within rows they are significantly different ($p<.05$).

[2]Superscripts following differences correspond to mean value for raw minus cooked. When designated by different superscripts within rows they are significantly different ($p<.05$).

[3]Not cooked due to residual hexane.

[4]Not replicated.

Of the washing treatments only the one using $NaHCO_3$ was significantly different for overall "L" value ($p<.05$) and showed comparatively little whitening when cooked ($p<.05$). This could be explained by the effect of higher pH on hydration and color previously discussed. Although some free water is lost during cooking more water may remain bound between swollen proteins than at lower pH thereby decreasing the amount of light reflected by the protein and by free water (Briskey and Kauffman, 1971).

The NaCl treatment was whitened by cooking significantly more than was the plus-fat treatment ($p<.05$). Lower precook heme-protein concentration in the plus-fat treatment (suggested by smaller "a" value) may have been partially responsible. Also, small cook losses encountered by NaCl treated mince compared to the plus-fat and plain water wash possibly suggests that free or surface bound water was available to return incident light (Table 14).

The H_2O_2 treatment was darker overall than the other treatments ($p<.05$) and was the only sample tested that darkened when cooked ($p<.05$). Just as increasing the pH may darken, decreasing the pH may lighten. However, upon adjustment to pH 6.0 the H_2O_2 treatment was noticeably granular and watery in appearance. This indicates protein aggregation and loss of functionality due to the shift toward the isoelectric point of the proteins. Moledina et al. (1977) found that mechanically deboned flounder appeared grainy and chalky and possessed poorer water holding capacity if acidulated to a pH less than 6.3. James and McCrudden (1976) did not report this condition for minced cod at pH 6.0 which may have a lower mean

isoelectric point than flounder or carp proteins. H_2O_2 treated carp was also exposed to very alkaline conditions (pH 10.5 or locally higher before NaOH was completely distributed) and partial hydrolysis of protein was likely. Setty et al. (1974) partially hydrolyzed flaked fish to produce a product lacking cohesiveness.

Cook yield was apparently lower than the control for the H_2O_2 treatment (Table 14) and some of the moisture retained was probably due to the presence of sodium tripolyphosphate. The very low cook yields determined for the water wash and plus-fat was probably related to high initial free water content rather than reduced protein functionality. A similar observation and conclusion was reported by Patashnik et al. (1976) for washed black rockfish mince. Significantly greater overall content of yellow pigment ("b" value) and an increase of red color ("a" value) after cooking (different than control and other treatments, $p<.05$) supports the contention that the color of H_2O_2 treated carp is determined more by free and oxidized porphyrins or products of carbonyl-amine reactions than by heat labile native pigments. All samples were stored frozen 10 weeks prior to analysis and yellow pigments associated with lipid oxidation may have developed and then concentrated during cooking. This is supported by the finding of elevated TBA numbers early in storage.

Among the control and treatments only the $NaHCO_3$ treatment lost shear resistance when cooked (significantly different, $p<.05$); all other treatments increased shear resistance. It has been well established that myofibrillar proteins generally toughen during

cooking perhaps by a combination of dehydration and the heat induced formation of strong bonds, including disulfide linkages (Asghar and Pearson, 1980, Lanier et al. 1980). The soft texture of the cooked $NaHCO_3$ treatment suggests that water of hydration and electronegative repulsion inhibited the development of these bonds. Even when total water content (free and bound) was nearly constant, Love (1975) detected texture changes in cod fillets that varied by as little as 0.1 pH unit.

The plain water, plus-fat and NaCl treatments increased shear resistance upon cooking significantly more than did the control ($p<.05$). When surplus moisture was evaporated or excluded from the tissue a larger percentage of protein as myofibrillar protein may have improved binding strength in these washes. Apparently increased water holding capacity in the NaCl treatment was responsible for less cook toughening than water wash and plus-fat treatments (significant, $p<.05$). Addition of vegetable fat had no significant effect compared to the water wash.

Elution of proteolytic enzymes with the wash water may be as important as myofibrillar protein concentration in increasing the firmness of wash treatments. Lin et al. (1980) and Lin and Lanier (1980) have shown that an alkaline protease in Atlantic croaker is very active at 50-70°C. Although not readily removed, some reduction in the activity of this enzyme was possible by washing minced fish with water (Lanier et al., 1980). During cooking then, more enzymatic proteolysis may have occurred in the control than in flesh from the water, plus-fat and NaCl washes.

Characteristics of muscle enzymes may also help to explain the results attained for the $NaHCO_3$ and H_2O_2 treatments. Lin et al. (1980) found a sharp increase in alkaline protease activity from very little at pH 7.0 to its maxima at pH 8.0. With a mean pH of 7.76 considerable softening of the $NaHCO_3$ treatment may have resulted when heated. The optimum pH for muscle acid protease (cathepsin) activity varied depending on substrate but most activity occurred below the pH 6.0 of the H_2O_2 treatment. Also treatment with H_2O_2 and NaOH may have denatured the enzymes as well as myofibrillar proteins.

Sensory Evaluation

When asked to rank samples in order of color intensity judgments of panelists were consistent with Hunter "L" values reported for cooked treatments (Table 15). All judgments indicated that the control and H_2O_2 treatments were darker than the 4 washes ($p<.01$), and the control was darker than the H_2O_2 treatment ($p<.01$). The $NaHCO_3$ treatment was darker than the other washes ($p<.01$). Due to residual solvent the hexane treatment was not sensory evaluated.

Color preference ranks were more variable but lighter samples were preferred. Except for the plus-fat samples the wash treatments were preferred to H_2O_2 ($p<.01$) which was preferred to the control ($p<.01$). Although darker than the other washes the $NaHCO_3$ treatment preference for color seemed not to hinge on color intensity. Perhaps the plumper, more acceptable appearance had an overriding influence. The wash treatments were not significantly different.

TABLE 15.--Sensory rankings of treated minced carp, 49 judgments.

Treatment	Characteristic (mean rank)						
	Color[1]	Color pref.[2]	Flavor[3]	Flavor pref.[2]	Texture[4]	Texture pref.[2]	Overall pref.[5]
Control	1.00[a]	5.65[a]	2.37[a]	3.20[a]	4.46[a]	4.24[a]	3.75[a]
Water wash	5.02[b]	3.02[b]	4.31[b]	3.61[a]	2.63[b]	2.98[b]	3.47[a]
Water wash plus fat	4.84[b]	3.35[bc]	4.80[bd]	2.94[ab]	2.75[b]	2.98[b]	2.86[b]
NaCl wash	4.65[b]	2.51[b]	3.96[bc]	2.71[b]	2.92[b]	2.71[b]	2.24[b]
$NaHCO_3$ wash	3.20[c]	2.67[b]	4.51[b]	3.31[a]	5.02[a]	4.02[ab]	3.31[a]
H_2O_2	2.00[d]	3.94[c]	1.06[e]	5.39[c]	3.00[b]	4.02[ab]	5.35[c]

[abcde]Ranks within columns with different superscripts are significantly different ($P<.01$).

[1]1 = darkest, 6 = lightest

[2]1 = most preferred, 6 = least preferred

[3]1 = strongest flavor, 6 = weakest flavor

[4]1 = firmest, 6 = softest

[5]1 = like best, 6 = like least

Flavor intensity of the H_2O_2 treatment was ranked significantly stronger than the control and other treatments ($p<.01$) and the control was stronger than the wash treatments ($p<.01$). The plus-fat sample was significantly stronger than the NaCl treatment possibly resulting from a dilution effect associated with a higher water content in NaCl samples. King (1974) concluded that washing minced fish with water could dilute flavor. However Ravichander and Keay (1976) were unable to determine a flavor difference between washed and unwashed minced fish presented as fingers to a taste panel. Variability in species flavor intensity, washing and fat removal procedures and product form could account for conflicting results. Washing reduced flavor intensity of minced carp in this study which was probably influenced by removal of both fat soluble and water soluble constituents.

Flavor preference did not necessarily follow intensity judgments. The NaCl treatment was the only wash that was preferred to the control ($p<.01$). Again, this may be related to moisture retention and presence of NaCl. At the low concentration present, it is doubtful that NaCl could be detected by panelists. However flavor perception of foods may be altered at a much smaller concentration of NaCl than its recognition threshold (Nicholas, 1979). The flavor preference for the NaCl treatment may be related to ionic dissolution of off-flavored organic compounds (the primary objective of this procedure) however flavor intensity was not ranked less than that of the other wash treatments. Miyauchi et al.

(1975) reported improved flavor scores for washed compared to unwashed minced fish only after 4 months of cold storage.

The results indicate that among these panelists the intrinsic flavor of minced Lake Huron carp is not objectionable. On a score sheet one panelist of Indian origin wrote the comment: "I like strong flavored fish." The presence of judges from differing cultural backgrounds may severely limit the applicability of preference data to U.S. consumers.

The judges rated the H_2O_2 treatment least preferred for flavor ($p<.01$). Ravichander and Keay (1976) did not use a formal sensory panel to evaluate H_2O_2 treated minced fish but personally found it to have a "very unpleasant flavour."

Texture of the water, plus-fat and NaCl treatments was judged firmer and preferred to the control ($p<.01$). The $NaHCO_3$ treatment was considered softer than the other washes ($p<.01$), was not significantly different from the control and was neither more or less preferred to the control or other treatment. These data for texture agree with shear press results reported previously suggesting that shear force values may be a suitable measure of firmness in cooked washed or unwashed minced carp. Similarly Wong et al. (1978) reported good agreement between sensory evaluation and an objective measure of compression resistance. Their study also showed an increase in firmness when minced fish flesh was water washed.

The H_2O_2 treatment was ranked significantly firmer than the control or $NaHCO_3$ treatment ($p<.01$) but not different from the

plain water, plus-fat or NaCl washes. The shear resistance of the H_2O_2 treatment was previously shown to be greater than that of all the wash treatments but the apparent contradiction is probably explained by the loss of protein functionality. H_2O_2 treated flesh seemed to possess less elasticity and was more crumbly and chewy than the other samples (author's judgment). The texture of the H_2O_2 and $NaHCO_3$ treatments were apparently less acceptable than the plain water, plus-fat and NaCl treatments but the differences were not statistically significant ($p<.01$). Ravichander and Keay (1976) and King (1974) expressed the opinion that H_2O_2 treatment is detrimental to minced fish texture although neither described the effect on texture.

The plus-fat and NaCl treatments were significantly preferred overall to the other treatments ($p<.01$). Based on the results of color, flavor and texture preferences the high overall preference ranking of these two treatments indicates that judges were more influenced by flavor quality than by color and texture characteristics. This was evidenced by the finding that the plain water wash was not different than the control for overall preference although it was significantly preferred to the control for color and texture. Similarly the $NaHCO_3$ treatment was not preferred overall to the control despite its highly preferred color, and the H_2O_2 treated mince was least preferred despite having a color preferred to the control.

Corrective Treatments - Conclusions

Washing minced carp with water shows promise as a means of improving color, texture and frozen storage stability, however processing yield and costs will dictate economic feasibility. Research is required to assess the functionality of washed minced carp in processed products since the need for washing may depend on end use. There was generally little benefit derived from the NaCl, $NaHCO_3$ and plus-fat modifications but the antioxidant effect of the $NaHCO_3$ wash warrants additional investigation. Hydrogen peroxide and hexane extraction treatments as prepared in this study are not recommended.

CHAPTER V

SUMMARY

Carp caught in Saginaw Bay, Lake Huron were mechanically deboned and subjected to 3 topics of study:

1. efficiency of deboning operation and characteristics of minced flesh.
2. effect of 2 commercial blend antioxidants on lipid stability during frozen storage and,
3. effect of 6 treatments designed to lighten color and reduce flavor on fresh, frozen and cooked characteristics.

The results of these investigations are reported below.

1. Passage of minced carp through the deboning machine for a second time appreciably reduced bone and scale concentration but was not deemed necessary or desirable due to small initial bone and scale content and to an apparent loss of textural integrity and elevated TBA value.

A machine yield of 42% of whole fish weight was lower than typically reported yields for many fish species perhaps because of heavy bones, skin and scales and the frequent occurrence of gravid females. Minced carp flesh was found to be highly pigmented and contained a larger percentage of fat than expected. Shear force resistance appeared to correlate with proximate composition where a high moisture, low fat batch of mince was firmer than a low moisture, high fat batch.

2. Lipid oxidation as measured by TBA analysis significantly increased after 6 months of frozen storage. A drop in TBA value at approximately 2 months of storage suggests that TBA is not a suitable index of lipid oxidation early during frozen storage of minced carp. Tenox 2 appeared to inhibit lipid oxidation more effectively than Freez-gard although they were not significantly reduced TBA values compared to the control only at the 12 month sampling date. Due to the apparent superiority of Tenox 2 as an antioxidant it was postulated that free radical chain termination was more effective than trace metal chelation at controlling lipid oxidation in minced carp. TBA numbers during frozen storage of carp were only slightly larger than values reported for sucker, a lean species (Morris and Dawson, 1979).

3. Washing minced carp or treating with H_2O_2 significantly reduced total heme pigment content compared to the control. The use of $NaHCO_3$ in the primary wash was apparently more effective at lowering hemeprotein content than a NaCl wash or a plain water wash, perhaps due to the effect of higher pH on globin conformation. Very low heme protein concentration was determined for washed mince that was supplemented with hydrogenated vegetable fat but this was partly credited to a difficulty with the pigment procedure. Hexane extraction did not significantly affect heme pigment content. As a condition of the procedures the wash treatments were dewatered until the plain water wash yielded 78-82% of original weight and the NaCl and $NaHCO_3$ washes yielded 72-78%. Except for the plus-fat treatment the washes were significantly higher in moisture and

all washes were lower in protein than the control. Although solublized protein concentration in wash waters was elevated in NaCl and $NaCHO_3$ washes differences were not significant.

The washes seemed to whiten and reduce redness but only the $NaHCO_3$ treatment was significantly less red than the control. The H_2O_2 treatment was no whiter than the washes but produced significantly smaller Hunter "a" and "b" values which may indicate pigment degradation and production of oxidized or free porphyrin compounds. Loss of yellow pigment following hexane extraction may have been caused by elution of carotenes.

The NaCl wash treatment and possibly the $NaHCO_3$ and plain water washes were more resistant to shear than was the control and plus-fat treatment. H_2O_2 and hexane extracted treatments did not affect raw texture.

Microbial load was not significantly affected by treatment, however the H_2O_2 procedure reduced total plate count 84%.

Bone and scale concentration was not significantly increased by washing. The $NaHCO_3$ treatment was the only "corrective" treatment with significantly different (smaller) TBA values during frozen storage. Although not statistically significant, all wash treatments probably improved frozen storage stability. The H_2O_2 and hexane treatments gave mixed results but did not improve lipid stability.

The control whitened (became gray) proportionately more upon cooking than did the treatments although it was darker after cooking than the washes. The $NaHCO_3$ treatment whitened the least

of the washes when cooked, possibly a pH effect, and the NaCl treatment whitened more than the plus-fat treatment, perhaps due to retained water. The H_2O_2 treatment was darker than the washes after cooking which may have been related to protein denaturation and to the presence of thermal stable pigments.

Cooking increased shear resistance in all wash treatments except for the higher pH, $NaHCO_3$ treatment. Concentration of myofibrillar proteins and removal of proteolytic enzymes were probably responsible. The H_2O_2 treatment toughened most when cooked which was related to protein denaturation and pH.

Results of sensory evaluation for color and texture generally agreed with objective measurements. All treatments that were evaluated improved color acceptability, most notably the plain water, NaCl and $NaHCO_3$ washes. The wash treatments reduced flavor intensity compared to unwashed mince but only the NaCl treatment was preferred for flavor. The H_2O_2 treatment was strongest flavored and least preferred of the treatments and control. Results indicated that intrinsic flavor of minced Lake Huron carp was not objectionable to most judges. The firmer textures of the water, NaCl and $NaHCO_3$ washes were preferred to the control. Overall the plus-fat and NaCl washes were preferred to the control and other treatments and the H_2O_2 treatment was least preferred. Flavor appeared to have a stronger influence on preference than color or texture.

APPENDICES

APPENDIX A

APPENDIX A.1.--Analysis of variance of the effect of Tenox 2, Freez-gard and untreated control on TBA-measured lipid oxidation during frozen storage.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	114.690	57.345	44.587	.001
Antioxidant	2	46.924	23.462	2.375	>.25
Residual	4	39.518	9.879	7.681	.01
Total	6	86.442	14.407	11.202	.01
Time	6	85.857	14.310	11.126	.01
Time x antioxidant	12	30.395	2.533	1.969	.10
Residual	34	43.729	1.286		
Total	52	159.981	3.077		
Grand total	60	361.113			

APPENDIX A.2.--Analysis of variance of difference values (control minus treatment) for the effect of Tenox 2, Freez-gard and untreated control on TBA-measured lipid oxidation during frozen storage.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	11.805	5.902	4.701	.05
Antioxidant	2	48.325	24.162	2.475	>.25
Residual	4	39.047	9.762	7.774	.01
Total	6	87.372	14.562	11.598	.01
Time	6	49.328	8.221	6.548	.01
Time x antioxidant	12	29.097	2.425	1.931	.10
Residual	34	42.691	1.256		
Total	52	121.116	2.329		
Grand total	60	220.292			

TABLE A.3.--Analysis of variance for proximate composition of "corrective" treatments.

Source of validation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Moisture					
Replication	2	692.29	346.14	7.554	.05
Treatment	6	2497.08	416.18	9.082	.01
Residual	9	412.40	45.82		
Total	15	2909.49	193.97		
Grand Total	17	3601.77			
Fat					
Replication	2	1627.09	813.55	11.270	.01
Treatment	6	13125.15	2187.52	30.303	.01
Residual	9	649.70	72.19		
Total	15	13774.85	918.32		
Grand Total	17	15401.94			
Protein					
Replication	2	561.77	280.89	3.450	.25
Treatment	6	6049.17	1008.20	12.382	.01
Residual	9	732.83	81.43		
Total	15	6782.00	452.13		
Grand Total	17	7343.77			

APPENDIX A.4.--Analysis of variance of the effect of "corrective" treatments on TBA-measured lipid oxidation during frozen storage.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	25.141	12.570	18.361	.01
Time	6	108.178	18.030	20.438	.01
Residual	12	10.586	0.882	1.289	>.25
Total	18	118.764	6.598	9.638	.01
Treatment	6	29.613	4.935	7.209	.01
Time x treatment	35	24.250	0.693	1.012	>.25
Residual	62	42.446	0.685		
Total	103	96.309	0.935		
Grand total	123	340.214			

APPENDIX A.5.--Analysis of variance of effect of cooking on Hunter L value.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	0.637	0.319	1.215	>.25
Form (cooked vs. raw)	1	182.475	182.475	5172.522	.001
Residual	2	0.071	0.035	0.135	>.25
Total	3	182.546	60.848	232.074	.01
Treatment	5	565.942	113.188	431.696	.01
Form x treatment	5	148.034	29.607	112.919	.01
Residual	20	5.244	0.262		
Total	30	719.220	23.974		
Grand Total	35	902.402			

APPENDIX A.6.--Analysis of variance of effect of cooking on Hunter a value.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	0.057	0.029	0.420	>.25
Form (cooked vs. raw)	1	102.684	102.684	2659.453	.001
Residual	2	0.077	0.039	0.567	>.25
Total	3	102.762	34.254	502.912	.01
Treatment	5	150.971	30.194	443.309	.01
Form x treatment	5	78.406	15.681	230.231	.01
Residual	20	1.362	0.068		
Total	30	230.740	7.691		
Grand Total	35	333.559			

APPENDIX A.7.--Analysis of variance of effect of cooking on Hunter b value.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	0.168	0.084	0.459	>.25
Form (cooked vs. raw)	1	23.847	23.847	144.832	.01
Residual	2	0.329	0.165	0.898	>.25
Total	3	24.176	8.059	43.930	.01
Treatment	5	35.098	7.020	38.266	.01
Form x treatment	5	0.279	0.056	0.304	>.25
Residual	20	3.669	0.183		
Total	30	39.046	1.301		
Grand Total	35	63.391			

APPENDIX A.8.--Analysis of variance of effect of cooking on shear force.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance of F
Replication	2	0.020	0.010	0.977	>.25
Form (cooked vs. raw)	1	32.013	32.013	3115.819	.001
Residual	2	0.021	0.010	1.024	>.25
Total	3	32.033	10.678	1063.742	.001
Treatment	5	23.226	4.645	462.757	.01
Form x treatment	5	20.117	4.023	400.818	.01
Residual	20	0.201	0.010		
Total	30	43.544	1.451		
Grand Total	35	75.597			

APPENDIX B

SCORE SHEETS USED FOR SENSORY EVALUATION

APPENDIX B

SCORE SHEETS USED FOR SENSORY EVALUATION

Fish Evaluation

Name_____ Booth #_____

Date_____

Taste the three samples as often as needed to determine Flavor differences. Two of these samples are the same, but one is different. Write the code of the samples which is different in the space below.

_____

Which of the samples do you Prefer for Flavor?

Same_____, Different _____, No preference _____, Check One.

Comments: _____

APPENDIX B.1.--Score sheet used to screen panelists.

FISH COLOR EVALUATION

NAME_____ DATE_____

BOOTH_____

INSPECT THE SAMPLES AND ARRANGE AS NEEDED TO DETERMINE COLOR DIFFERENCES. DO NOT TASTE THE SAMPLES.

RANK THE SAMPLES IN ORDER OF COLOR INTENSITY, AND WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	DARKEST
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	LIGHTEST

RANK THE SAMPLES IN ORDER OF COLOR PREFERENCE. WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	MOST PREFERED COLOR
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	LEAST PREFERED COLOR

COMMENTS_____

APPENDIX B.2.--Score sheet used to sensory evaluate color of "corrective" treatments.

FISH FLAVOR EVALUATION

NAME_____ DATE_____

BOOTH_____

TASTE THE SAMPLES AS OFTEN AS NEEDED AND IN ANY ORDER YOU WISH.
RANK THE SAMPLES IN ORDER OF FLAVOR INTENSITY,
AND WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	STRONGEST FLAVOR
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	WEAKEST FLAVOR

RANK THE SAMPLES IN ORDER OF FLAVOR PREFERENCE.
WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	MOST PREFERED FLAVOR
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	LEAST PREFERED FLAVOR

COMMENTS_____ _____

APPENDIX B.3.--Score sheet used to sensory evaluate flavor of "corrective" treatments.

FISH TEXTURE EVALUATION

NAME_____ DATE_____

BOOTH_____

TASTE THE SAMPLES AS OFTEN AS NEEDED AND IN ANY ORDER YOU WISH.
RANK THE SAMPLES IN ORDER OF TEXTURE FIRMNESS,
AND WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	FIRMEST
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	SOFTEST

RANK THE SAMPLES IN ORDER OF TEXTURE PREFERENCE.
WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	MOST PREFERED TEXTURE
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	LEAST PREFERED TEXTURE

COMMENTS_____

APPENDIX B.4.--Score sheet used to sensory evaluate texture of "corrective" treatments.

FISH ACCEPTABILITY

NAME_____ DATE_____

BOOTH_____

EVALUATE THE SAMPLES AS OFTEN AS NEEDED AND IN ANY ORDER YOU WISH.

RANK THE SAMPLES IN ORDER OF OVER-ALL PREFERENCE ACCORDING TO YOUR OWN STANDARDS.

WRITE THE SAMPLE CODES BELOW.

	CODE	
1	_____	LIKE BEST
2	_____	
3	_____	
4	_____	
5	_____	
6	_____	LIKE LEAST

COMMENTS_____

APPENDIX B.5.--Score sheet used to sensory evaluate over-all preferences of "corrective" treatments.

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