DEVELOPMENT OF A PROCESS FOR PREPARING A FISH PROTEIN CONCENTRATE WHICH CAN BE RECONSTITUTED INTO A MEAT-LIKE PRODUCT

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TAMU-SG-72-201

MARCH 1972

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Partially supported by the National Sea Grant Program
Department of Commerce Grant 2-35213
Texas A&M University

Sea Grant Publication No. TAMU-SG-72-201

March 1972

ABSTRACT

A process for preparing fish protein concentrate (FPC) with rehydration and emulsifying properties is described. Micropogon undulatus, which is discarded during shrimping operations in the Gulf of Mexico, is used as a starting material. Theoretical aspects of maintaining functional properties in hot solvent extracted proteins are discussed. The rehydration and emulsifying capacities of fish protein are maintained by pH adjustment to prevent protein-protein interaction during hot solvent extraction processes.

The process for preparing FPC from 100 g of fish muscle consists of the following steps: (1) comminution with 5 g of NaCl, (2) adjustment of pH to 2.5 with 1N HCl, (3) extraction of lipids with 400 ml of 1:1 mixture of 95 percent ethanol and hexane by refluxing at 70°C for 30 minutes, (4) removal of solvents by filtration or centrifugation, (5) repetition of the steps 3 and 4 until most of the lipids are removed and (6) drying of FPC below 70°C in air or vacuum.

The FPC produced by this method has the following qualities: (1) amino acid spectrum similar to that of the fish muscle, (2) bacteriologically sterile, (3) water retention capacity at least twice the capacity of the original fish muscle, (4) forms emulsions in oil-water mixtures, (5) has high protein efficiency ratio, (6) appears to be useful as a binder protein in sausage-like

products and (7) forms milk-like suspensions. A partially refined fish oil is produced as a co-product.

The process removes Pb, Cd and As from fish muscle but not Hg. The Hg in FPC is rapidly incorporated into and retained by the blood, kidneys and livers of rats.

ACKNOWLEDGEMENTS

The authors acknowledge with gratitude the contributions of all those who helped in preparation of this report. Special mention is made of the following persons and organizations who have significantly affected the final form of this report:

The Department of Biochemistry and Biophysics for making available the facilities during the feeding studies on rats;

Dr. E. M. Wright for his personal help in conducting the histopathological studies on rats;

Messers B. Berry, C.S. Custer, A.W. Matthys, C.A. Thompson, Jr., K.K. Sharma and Mrs. E.L. Wright for their help in performing various experiments;

The Animal Science Department and the Center for Marine Resources for providing the financial support; and

Dr. C.E. Gates, Dr. S.M. Gillespie, Mr. R.L. Hostetler and Dr. G.C. Smith for their help in conducting the organoleptic evaluation.

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CHAPTER I

INTRODUCTION

The specter of hunger and malnutrition, which has haunted mankind from ancient times, threatens to become acute over wide areas of the world. Food production must increase by 50 percent over the next 20 years to keep pace with growing population (CMSER, 1969)*. In this endeavor every food source must be utilized to the fullest extent.

The oceans and seas cover more than 70 percent of the surface of the earth and harbor untold quantities of protein materials. Yet, this abundance of wealth has not been fully exploited (Christy, 1967). Currently, the world's annual harvest of fish and other marine foods is about 57 million metric tons (FAO, 1970). Even if fishery production were not increased, direct utilization of all of this harvest for human consumption would result in a tremendous increase in edible protein. The increase would certainly be above the amount of edible protein which is now produced through the use of fish meal in poultry and animal feeds. Therefore, harvesting of unutilized and underutilized marine species and the development of products for direct human consumption is one of the most productive areas for solving the world food problem and alleviating the protein malnutrition in the

undernourished populations.

Fish is an established major source of animal protein in many countries of the world. In such countries, production of poultry and red meats has been low and/or expensive while fishery products have been readily available and relatively inexpensive. Japan, the Scandinavian countries and some of the Latin American countries have accepted fish as their staple meat. The highest annual per capita consumption of fish and fishery products on the basis of edible weight is about 28 kg in Japan and 21 kg in Sweden (Carsten, 1970). In contrast, in the United States where large amounts of other sources of protein have been available, the annual per capita consumption of fish is about 6 kg (Anon., 1967).

Various authors have estimated values ranging from 55 to 2000 million metric tons for the annual sustainable fisheries harvest of the world (Chapman, 1966; Graham and Edwards, 1962; Larkin, 1965; Schmitt, 1965). In the United States, an estimate of the unutilized and underutilized fish stocks in coastal waters was reported recently to be 660,000 metric tons (Bullis and Carpenter, 1968). These unwanted fish are killed and discarded every year during shrimping operations (Moffet, 1967).

Many of the so-called "trash fish" or "industrial fish" caught during shrimping operations have no practical market now except as fertilizer, pet food or fish meal and are, therefore, not available for human consumption. The utilization of these fish depends upon the development of efficient harvest and preservation methods and on

the development of products acceptable for human consumption.

Some of the areas where technological advancement and innovation are needed for the development of inexpensive and wholesome products from the industrial fish taken in shrimping operations may be summarized as follows:

1. Post-harvest storage under improved, inexpensive methods of refrigeration until processing
2. Development of more efficient and economical methods of cleaning, evisceration and deboning of various species and sizes of fish
3. Development of methods for the removal of odoriferous and obnoxious compounds from the fish flesh
4. Development of procedures for the extraction of protein and nutritious compounds for use in food products
5. Formulation of products for utilizing deboned fish and other extracted compounds from fish.

Product development itself presents a big challenge because traditionally fish has been considered for consumption as close to its original form as possible. Unconventional products would require exceptional food attributes to compete against the available conventional products of plant and animal origin.

The recent developments of defatted, dehydrated fish flour in the United States, Canada and Sweden have provided a high quality protein powder from "trash fish" for use as a supplement in the conventional food items (Carsten, 1970). This fish flour, commonly known as fish protein concentrate (FPC), has an excellent amino acid balance (BCF., 1966). It is considered to be ideal for alleviating protein malnutrition (Finch, 1969). However, it cannot be used as a

food, per se, because of its poor solubility and rehydration properties. Studies on the solubilization of FPC are in progress (Cheftel et al., 1971). However, because of the lack of rehydration capacity and the persistance of fish flavors, little progress has been made in these studies. Therefore, research for improvement of the functional properties of FPC without impairing the nutritional qualities could lead to the utilization of FPC as a food rather than a food supplement.

In this study, the principles of defatting and dehydration have been applied to Micropogon undulatus, the species available in abundance in the Gulf of Mexico, for the preparation of a protein concentrate of high nutritional quality which can be rehydrated and emulsified. To achieve this, modifications in process variables such as pH, temperature and solvent system have been made.

The protein concentrate thus produced has been evaluated for its qualities as follows:

1. Nutritional: amino acid content and protein efficiency ratio
2. Bacteriological: presence of pathogenic microorganisms and putrefactive anaerobes and enumeration of aerobic and anaerobic bacteria
3. Toxicological: presence of heavy metal residues and histopathological examination of rats fed on the protein concentrate as sole source of protein
4. Functional: rehydration and emulsifying properties and its use in sausage as a replacement for beef protein

5. Organoleptic: (a) color and odor of the protein concentrate
(b) color, odor and texture of the sausage product made with the protein concentrate.

CHAPTER II

REVIEW OF LITERATURE

Historical background:

The problem of utilization of unwanted or "trash fish" in commercial harvest was recognized in the beginning of the century in the United States. A report published in 1907 suggested the utilization of those species which were not marketable (Field, 1907). From 1931-35, the U. S. Bureau of Fisheries recorded the type and number of fishes captured incidentally in shrimp trawling operations on the south Atlantic coast (Anderson, 1968). During the period from 1950 to 1970, several authors reviewed the availability and possible use of trash fish for pet food manufacture (Anderson, 1968; Compton, 1969; Haskell, 1961; Moffet, 1967; Roithmayr, 1965a). Bullis and Carpenter (1968) in a review of the latent fishery resources reported the production of over 45,300 metric tons of trash fish in the Gulf of Mexico. They also reported that in addition to the 1967 yield of 48,762 metric tons of "trash fish" or "industrial bottom fish," an estimated additional 660,317 metric tons of trash fish were caught and discarded during Gulf shrimping operations. A recent survey has shown that the unutilized fish which are killed and discarded from shrimp vessels on the Texas coast of the Gulf of Mexico amounted to 265,143 metric tons per year (Moffet, 1967).

Status of utilization:

Although large quantities of "industrial" fish are killed and discarded by shrimpers, some are presently utilized as food fish and some as pet food and fish meal. In 1952 a pet food industry, utilizing trawl fish as raw material, was started in Mississippi (Roithmayr, 1965a). By 1962 about 85 percent of the catch of 48,762 metric tons of trash fish, landed in the Mississippi-Louisiana area, was utilized as canned pet food (Roithmayr, 1965a). Reduction of fish for feed, fertilizer and oil production has proven to be another way of utilizing industrial fish. During the last 30 years rapid growth of the fish meal industry for animal feed production has consumed large amounts of trash fish (Mead, 1971). Currently about 43 percent of the world fish production is used in making fish meal (Vickery, 1971).

Constitution of catch and potential yield:

Most of the surveys of trawl fish along the Gulf and Atlantic coasts have revealed an abundance of four species of the Scienideae or drum family (Anderson, 1968; Haskell, 1961; Roithmayr, 1965a). Bullis and Carpenter (1968) have estimated expansion of the bottom fishery in Gulf and Southeast Atlantic coasts to be 88 times the 1968 catch of 46,050 metric tons consisting mostly of Micropogon undulatus (croaker), Leiostomus xanthurus (spot) and Cynoscion regalis (sea trout). Of these, M. undulatus constituted 50 to 55 percent of the total catch by weight (Anderson, 1968; Haskell, 1961). The 1962

figures showed a record production of 29,053 metric tons of M. undulatus in the Gulf of Mexico (Roithmayr, 1965b). A very recent survey shows that this species is still estimated to be available in the Gulf of Mexico at the rate of 30 to 50 percent of the total catch taken at about a 10-fathom depth where most shrimping takes place (Compton, 1969).

Cause of waste:

At present the pet food, fish meal and fertilizer industries utilize those industrial fish which are landed by special boats while those caught by shrimp boats are usually dumped. Finch (1970) has analyzed the cause of discarding of fish caught during shrimping operations. Cobb (1970) has recently summarized the reasons from the fisherman's point of view which are as follows:

1. Small size and variety of fish
2. High cost of storage under mechanical refrigeration and
3. No demand for the fish at prices required by the shrimpers to land them.

Gross composition of M. undulatus:

Thompson (1959a, 1959b, 1959c) has measured the proximate composition of M. undulatus caught in the Gulf of Mexico during different seasons. Rahman (1970) has reported the proximate analysis of M. undulatus caught from the Galveston Bay. On the average M. undulatus has 16 percent protein, 4.5 percent oil, 4.5 percent ash and 75 percent moisture (Thompson, 1959a; 1959b; 1959c).

However, some M. undulatus contained as high as 10 percent oil (Thompson, 1959b). The protein content varied from 16.5 percent to 17.1 percent (Thompson, 1959c). The ash content varied from 2.37 percent to 6.42 percent (Thompson, 1959c). The range of moisture content was from 67.1 percent to 78.5 percent (Thompson, 1959c).

Possible means of utilization:

The idea of using comminuted fish for making products for human consumption is not new. Vickery (1971) has supported this idea for making a variety of products to increase seafood consumption. Many products from comminuted fish have already been successfully marketed. In Japan, fish sausages and 'hams" have already become conventional seafood products. The annual production of fish sausage and "ham" in Japan had grown from 81,269 metric tons in 1959 to 170,660 metric tons in 1964 (Nair, 1968; Tanikawa, 1968). In the United States, tuna fish sausage was sold commercially in 1958 (Finch, 1970). In India, fish sausage is considered an acceptable product (Krishnaswamy et al., 1964). Fish fingers developed in the United Kingdom have been very successful (Coburn, 1969). Recently in Japan a number of new products have been successfully prepared and marketed (Anon., 1967). Most of these comminuted fish products are prepared from large fish which can be eviscerated, skinned and deboned. Utilization of small fish in this manner would not be possible unless efficient flesh separating devices are invented for small fish.

Large M. undulatus species are used as food fish. Some are used

in pet food manufacture. An expansion in the landing of M. undulatus would provide the large fish as raw materials for making new comminuted seafood items. However, the small M. undulatus species having equally good food attributes do not have a place in the food market. The small fish present the problems of efficient evisceration, descaling and deboning. Any process devised to solve these problems of dressing the small fish would yield materials of high protein and intermediate oil content. Further processing would then be required to prevent bacterial spoilage and deterioration due to oxidation of unsaturated lipids. The meat produced in this manner could then be utilized as a base for developing new products for human consumption.

Fish meal and oil:

Fish meal and the accompanying fish oil production are the major diversion of world fishery resources from direct human consumption. In 1968 the world production of fish meal was 5,080,000 metric tons (Meade, 1971). Fish meal, while serving as an excellent source of protein in animal feed, is an inefficient method of protein utilization. Roels (1969) has expressed this by suggesting that 1,000 kg of fish reduced to fish meal for animal feed would produce 1 kg of protein in man. If the same 1,000 kg of fish were consumed directly, it would produce 10 kg of protein in man. Therefore, conversion of fish protein via fish meal to edible protein has an efficiency of only 10:1. For poultry feed the efficiency is 6:1 (Byerly, 1967). This has led to the development of processes for the conversion of fish

meal and whole fish into protein concentrates for direct human consumption. However, these protein concentrates usually retain strong fishy flavors and are not readily accepted.

Fish protein concentrate (FPC):

The term "fish protein concentrate' (FPC) was adopted by FAO in 1961 in preference to the earlier name 'fish flour" to avoid confusion with cereal flours which it resembles in general physical form but not in chemical or food formulating properties (Anon., 1962). Pariser(1967) has defined FPC as an inexpensive, stable, wholesome product of high nutritive qualities, prepared for human consumption from whole edible fish by sanitary food processing methods. He adds that FPC, according to his definition, is a product that is more concentrated in protein and certain other components of nutritional importance than is the raw material from which it is prepared. In present usage, however, the term FPC is generally restricted to a solvent-extracted product, high in nutritive value, and low in functionality. Moreover, the concept of using whole fish as raw material has changed to include eviscerated, skinned and deboned fish also.

Numerous processes for the production of FPC have been listed and described by Noyes (1969) and Finch (1970). Patents in the United States, Canada, the United Kingdom and other countries have been granted for many of these processes. These range from simple methods of solvent extraction of fish meal to rather sophisticated methods

of preparing relatively pure proteins is iso-electric precipitation from alkali or acid solubilized fish (Finch, 1970).

Until now three methods have been developed to produce FPC commercially. These are discussed briefly.

1. Viobin Process: In this process comminuted fish is dehydrated by an azeotropic distillation of the water with ethylene dichloride to remove lipids and is then vacuum-dried to remove residual solvent (Levin, 1959).

2. Isopropanol Extraction Process: This process was first proposed in Canada (Guttman and Vandenheuvel, 1957) but developed for commercial production in the United States (BCF, 1966). The process utilizes a successive series of extractions with azeotropic isopropanol (IPA) moving countercurrent to the product to both dehydrate and defat comminuted fish. Most of the residual solvent is then removed by either vacuum or atmospheric drying. Steam stripping has been necessary for complete removal of solvent from the FPC.

3. Astra Process: This process is under development for commercial production of FPC in Sweden (Anon., 1970). An aqueous-solvent combination is utilized during processing. A wet press cake is produced from heat-processed and deboned fish. Residual lipids are removed by continuous extraction of the press cakes with alcohols. The extracted press cake is then desolventized and steam dried (Somlai, 1967; Lawler, 1970).

All of the three processes result in FPC of high nutritional quality. However, two of the processes do not result in the recovery

of high quality fish oil. In the Viobin process the azeotropic solvent produces oil of poor characteristics. Another disadvantage of this process is the toxicity of the solvent which has to be removed by further extraction with alcohol. The isopropanol extraction process also results in a poor quality oil. The other problem in this process is the codistillation of organic volatiles like amino acid carbonyls with the alcohol-water azeotrope. This is being solved now by using acidification of the alcohol-water azeotrope prior to distillation. In the Astra process it is expected that oil recovery would be no problem. A lower yield of protein would, however, be expected due to the losses of soluble proteins and proteins adhering to the bones and skin.

None of the commercially feasible processes produce FPC with high "functional properties." Since the material cannot be used as a food, per se, and does not possess flavoring, texturizer, binder or preservative properties, it must be used as a supplement in such foods as breads, pastas, cereals and cookies (Sidwell, 1967). Its extremely low solubility appears to exclude it from incorporation into beverages. However, solubilization studies for the development of high protein content beverages are still in progress (Hale, 1969; Roels, 1969; Tannenbaum et al., 1970; Cheftel et al., 1971).

Extraction of proteins from fish:

Several authors have extensively reviewed the composition and characteristics of fish proteins (Hamoir, 1955; Connell, 1962; Buttkus,

1963). The myofibrillar proteins consisting of actin, myosin and tropomyosin constitute 80 percent of the muscle proteins. Of these, the complex of actin and myosin called actomyosin accounts for 67 to 75 percent of the total muscle proteins. Connell (1962) has considered actomyosin, the main fraction of the myofibrils, to be responsible for the gel-like properties of the muscle. Any process of the extraction of proteins from fish should, therefore, be devised for maximum extraction of actomyosin as close to its native form as possible.

Dyer et al. (1950) have demonstrated that blending fish muscle in a 3 to 5 percent NaCl solution at 5°C and at pH 7 to 9 extracted up to 95 percent of the fish muscle proteins. However, below pH 5.0 almost all of the proteins were insoluble in 5 percent NaCl solution. Rahman (1970) has recently showed that only 14 percent of the proteins of M. undulatus were soluble at pH 6.0 in the presence of 3 percent NaCl. However, the protein solubility increased to approximately 60 percent at pH 3.5 and then decreased to 45 percent at pH 2.0. These observations suggest that a process could be developed for the extraction and purification of the protein from the descaled, eviscerated and deheaded M. undulatus.

CHAPTER III

THEORY AND DEVELOPMENT OF PROCESS

Preliminary experiments for the development of a sausage-like product from comminuted fresh fish indicated the necessity of prior removal of lipids and odoriferous compounds. The details of these experiments are described in Chapter IV. Lipid oxidation in the products could be controlled by the addition of antioxidants and/or liquid smoke (Watts and Faulkner, 1954). However, fishy odors, which were evident in all the products, could not be masked by smoke or spices. Petroleum and obnoxious odors were also evident in the products made from fish taken from Galveston Bay. The low standard plate count, below 10^4 microorganisms per gram of comminuted fish, suggested that the tainting of fish flesh was not caused by bacteriological deterioration, but was due to chemical agents present in the polluted waters of Galveston Bay.

The next step was an attempt to remove the odoriferous compounds by an aqueous solvent extraction process at low temperatures (5°C and 25°C) and then by an ethanol extraction process at the same low temperatures. Since these processes, although showing promise for less odoriferous fish, were unsuccessful, the next logical step was to employ a hot solvent extraction process. Described below is the theory and development of the process.

Preparation of FPC by extraction at neutral or slightly acid pH:

Ample evidence exists that in the normal or undenatured state, the protein molecule is arranged in such a manner that hydrophilic or ionic groups are oriented toward the aqueous environment while hydrophobic groups are oriented toward the interior of the molecule (Butler, 1971). This is illustrated schematically in Figure 1A.

During the various hot solvent extraction processes which have been proposed for the preparation of FPC (Levin, 1959; Pariser and Odland, 1963; BCF, 1966), the protein molecule is exposed to a more hydrophobic solvent medium (ethanol, isopropanol, ethylene dichloride) than its normal aqueous environment. The heat energy is sufficient to break hydrogen and hydrophobic bonds which help maintain the structure of the molecule. The resulting denatured molecules react to form micelles with some of the hydrophobic side chains oriented toward the exterior of the micelle (Figure 1B). The more hydrophobic the solvent medium, the greater the reorientation. Studies on the denaturation of spermwhale myoglobin have demonstrated this phenomenon (Herskovits and Jaillet, 1969). The reorientation of the hydrophobic groups would result in a corresponding orientation of the hydrophobic groups toward the interior of the micelle. At pH values where large numbers of positive and negative charges are remaining on the protein molecules, the micelle is stabilized by the formation of relatively high energy ionic bonds. When the micelle is dried, the hydrophobic groups form a surface barrier to the penetration of water.

The evidence for this mechanism is the sand-like character of

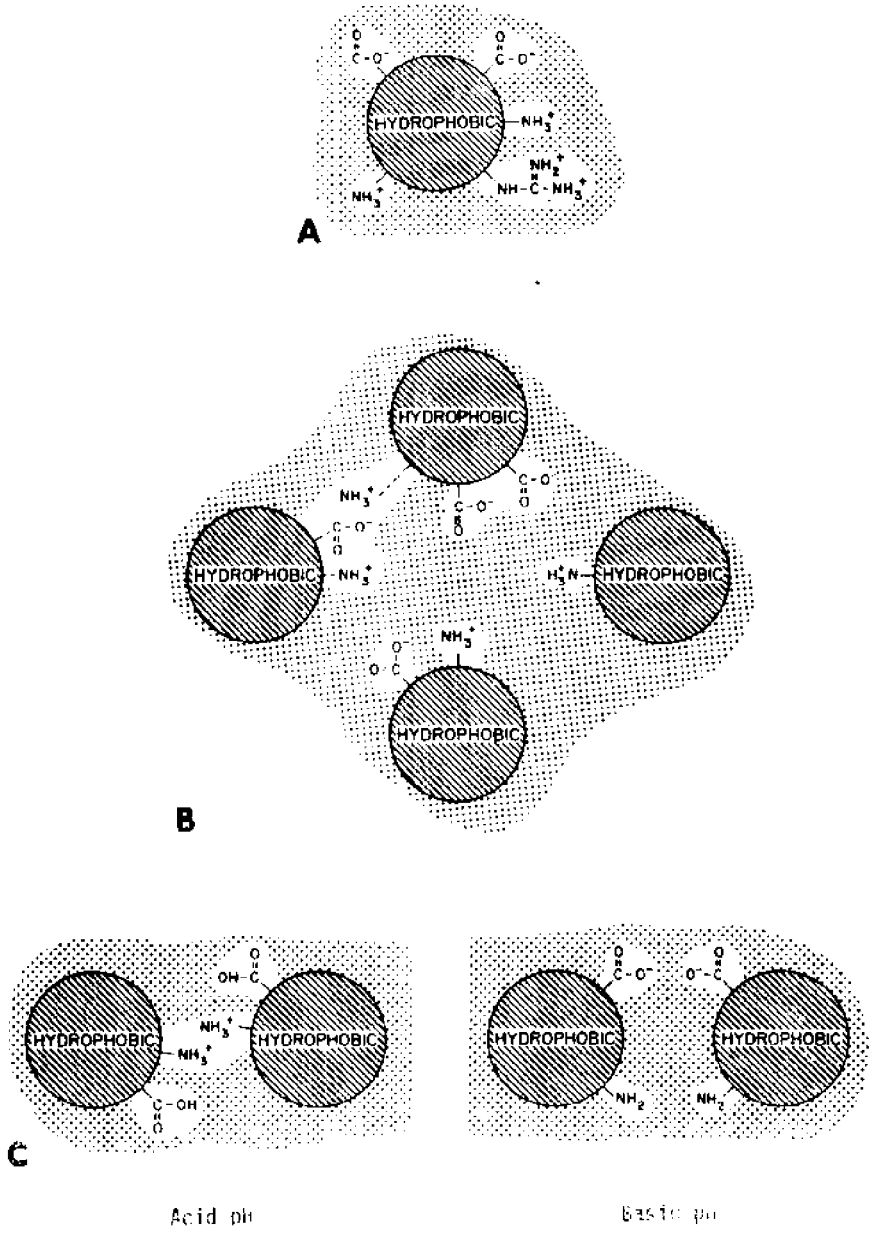


Fig. 1 - Schematic diagram of protein micelle. (A) Hydrophobic groups oriented toward the interior of the molecule - normal or undenatured state; (B) Hydrophobic side chains oriented toward the exterior in organic solvents - denatured state; and (C) Reduction of micelle formation due to pH adjustment - like charges would repel each other.

acids; 2) it is toxic, necessitating complete removal. Ethanol which is non-toxic at low levels and is relatively unreactive with acids was chosen as the primary solvent.

Fish proteins tend to form gels in the ethanol water mixtures which are present in the early stages of extraction. Protein gels form at acid pH and low ionic strength because of electrostatic repulsion due to the positively charged protein molecules (Figure 2A). There also appears to be a corresponding swelling of the molecules which makes solvent removal difficult. Studies with a model compound, DEAE-sephadex, indicate that the electrostatic repulsion of high molecular weight molecules can be greatly reduced by the addition of neutral salt (Figure 2B). Once most of the water has been removed by solvent extraction the problem of gel formation no longer exists.

Preliminary evidence indicated that although 90 percent recoveries of protein could be obtained from lean fish, in fat fish the recovery decreased significantly. This appeared to be caused by the combination of a detergent effect of the lipid and the necessity for additional extractions to remove the lipid. To increase the efficiency of the solvent extraction, an equal volume of hexane was added.

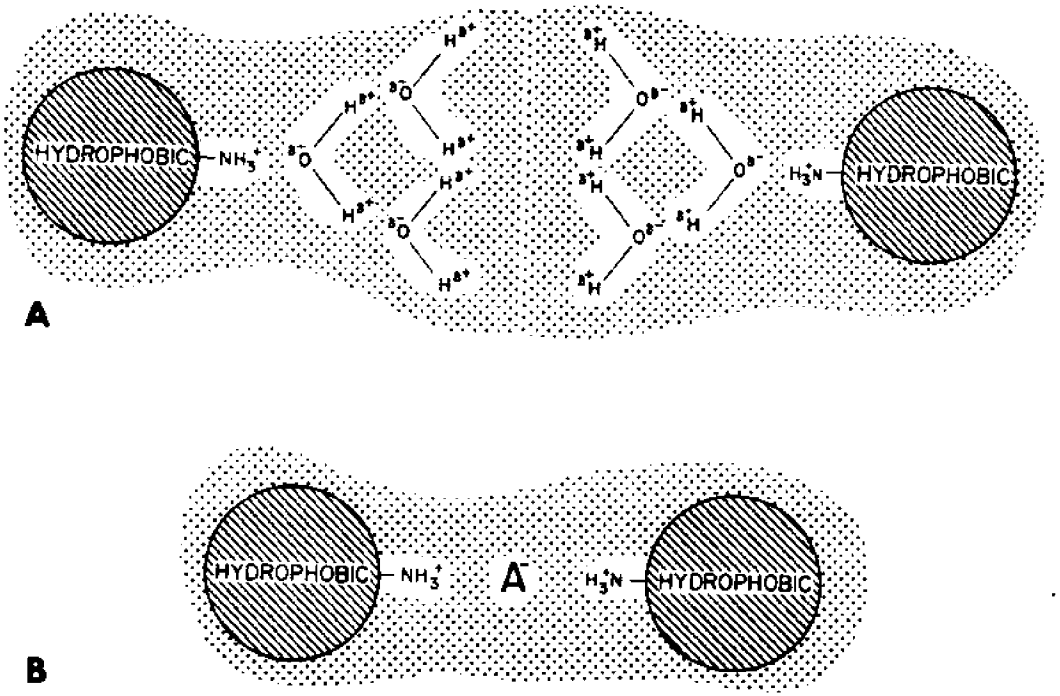


Fig. 2 - Schematic diagram of gel formation by protein micelle. (A) Protein gel at acid pH and low ionic strength due to electrostatic repulsion; and (B) Collapse of gel due to reduced electrostatic repulsion by the addition of neutral salt.

CHAPTER IV

EXPERIMENTAL

Materials:

Atlantic Croaker (Micropogon undulatus) 10 to 30 cm long were collected from boats during shrimp trawling operations in Galveston Bay and the Gulf of Mexico. The fish were packed in ice and transported to Texas A&M University. Scales, viscera and heads were removed in the laboratory. The dressed fish were glazed with distilled water and stored in plastic bags at -20°C. Ethanol (95 percent) was obtained from several commercial sources. Hexanes and diethyl ether were obtained from Fisher Scientific Company, Fair Lawn, New Jersey, U. S. A. The reagent grade "hexanes" were redistilled with the middle fraction being retained. The first and last 15 percent of the distillate were discarded to reduce off-flavored residues.

Preparation of partially deodorized protein:

(a) Preparation of protein pellets

A salt extraction and precipitation procedure for the preparation of protein pellets was developed on the basis of observations of Dyer et al. (1950), Rahman (1970) and Chu and Pigott (1970) that fish proteins are insoluble in 5 percent NaCl at low pH. The procedure is schematically explained in Figure 3. The 5 percent NaCl solution was chosen because at pH 2.5 flocculent precipitates were produced

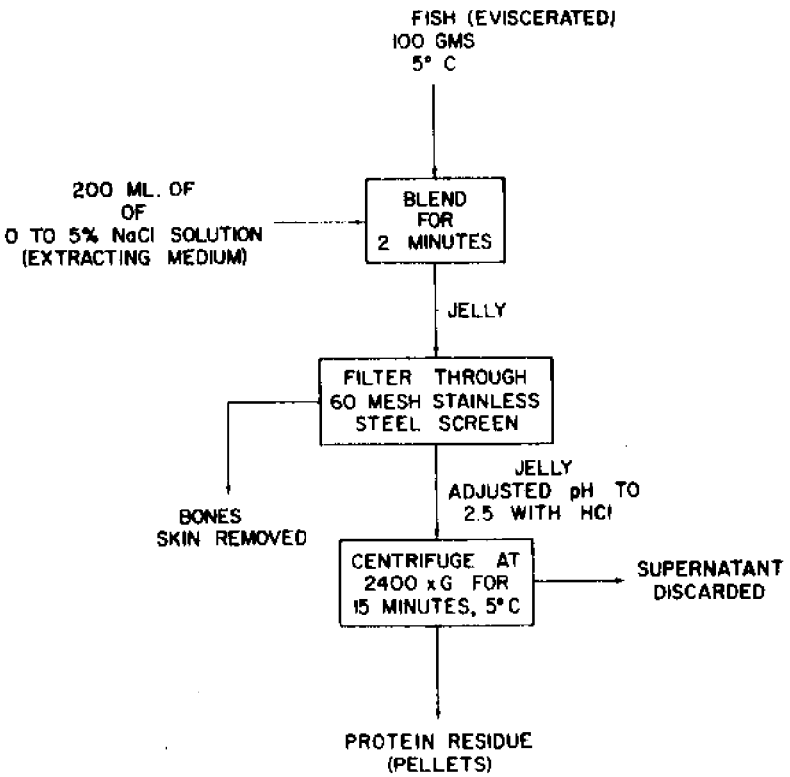


Fig. 3 - Flow diagram for the preparation of fish protein pellets.

which could be centrifuged to yield pellets with meaty character. The protein prepared by this procedure, however, needed washing for the removal of lipids and odoriferous compounds. Protein pellets were also prepared in a similar way by adding to the fish varying concentrations of NaCl, 1N HCl for adjusting pH and the solvents.

(b) Washing of pellets

Five washing solutions were selected to reduce the level of odoriferous compounds with minimum loss of protein during the washing cycle. The washing solutions were as follows:

(i) distilled water

(ii) 3 percent NaCl solution

(iii) 3 percent NaCl solution adjusted to pH 2.5 with 12N HCl

(iv) 95 percent ethanol

(v) 95 percent ethanol acidified with 1 percent 12N HCl.

The pellets were resuspended in 200 ml of washing solution and centrifuged at 2400 x G for 15 minutes at 5°C. A small portion of the supernatant was saved for protein analysis, the remainder discarded.

Preparation of FPC:

Fillets were removed from larger fish (above 20 cm long) and ground or minced into small pieces. Smaller fish were ground without removing the bones or scales. Sodium chloride (5 percent by weight) was added and the material was blended in a Hoover blender until a smooth jelly was obtained. The pH of the jelly was adjusted

to 2.5 with 1N HCl and 2 ml of ethanol per gram of protein were added. In an alternate procedure the ethanol was added and the pH of the resulting mixture was then adjusted to 2.5. The mixture was transferred to a round bottom extraction flask equipped with an efficient reflux condenser. Redistilled hexane equal to the volume of the ethanol was added. The mixture was rapidly stirred and heated in a water bath until the hexane began refluxing (approximately 70°C). After 30 minutes at reflux temperature the hexane was decanted and ethanol was removed by filtration or centrifugation. The protein was returned to the extraction flask and amounts of ethanol and hexane equal to the original volumes were added. The procedure was repeated from 2 to 4 times depending upon the fat content of the fish. Figure 4 presents the flow diagram of the process. A final ethanol rinse was necessary to remove odoriferous residues remaining from the hexane.

Preparation of sausage-like products:

(a) Product from fresh fish

Batches of the frozen fish were thawed under running tap water. They were comminuted in a meat grinder to reduce the bone size to less than 3.5 mm in length. The comminuted fish were chilled in an ice bath and held there until used. Standard plate counts of the comminuted fish were taken to ascertain that the bacterial load was below 10^4 microorganisms per gram. Ingredients of the sausage (Table 1) were manually mixed and then passed through a meat

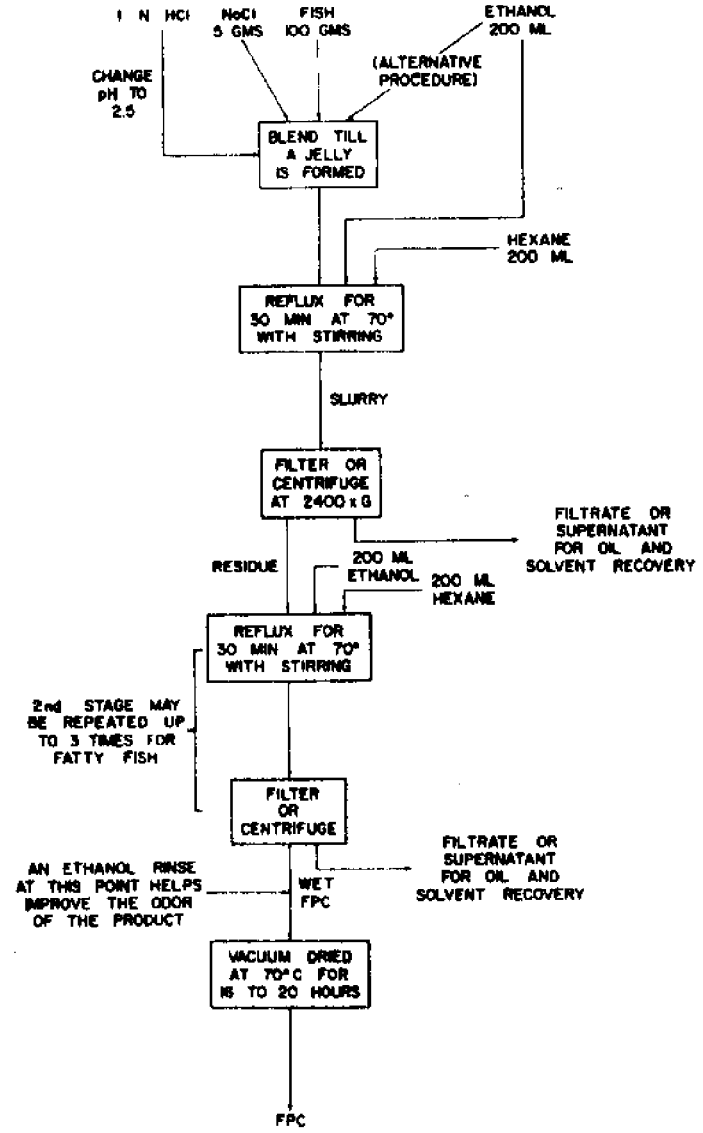


Fig. 4 - Flow diagram for the preparation of fish protein concentrate (FPC).

Table 1 - Ingredients in the sausage formulations

Ingredients	Types of sausage formulations (gm)			
	Fresh Fish	Control	FPC I[a]	FPC II[b]
Beef	-	1132.5	906.0	679.5
Pork	-	1132.5	1132.5	1132.5
Fish	2265	-	-	-
FPC	-	-	68.0	135.9
Ice[c]	-	906.0	1064.6	1223.1
Spice	113.25	11.4	11.4	11.4
Salt	45.3	62.5	62.5	62.5
Dextrose	-	15.5	15.5	15.5
Prague Powder	-	7.0	7.0	7.0
Shortening	113.25	-	-	-
Liquid smoke	15.0	-	-	-

[a]20 percent of beef replaced with rehydrated FPC.

[b]40 percent of beef replaced with rehydrated FPC.

[c]Extra ice was added to provide water of rehydration for FPC.

grinder at least twice to insure uniform mixing. The sausage emulsion was stuffed in edible collagen casing. Links of 15 cm length were made and cooked in 90°C water bath for 60 minutes to soften the bones. The sausages were then deep fat fried in vegetable shortening to give a bright brown color.

(b) Product from FPC

Sausage-like products were formulated by substituting rehydrated FPC (4 parts water, 1 part FPC) for 20 and 40 percent of the beef in a wiener type emulsion (Table 1). The FPC was added in a dry form and rehydrated in situ. Extra ice was added to provide the water of rehydration. The formulations are reported in Table 1.

Lean beef and pork trimmings were finely ground in a meat grinder and passed through a plate having perforations of 3 mm. All the ground beef and half the ice were chopped and mixed in a silent cutter for 2 minutes. Pork trimming, FPC, spices and the remainder of the ice were then added to the ground beef. The chopping and mixing were continued for at least 8 minutes or until the temperature of the emulsion had reached 12.8°C. The emulsion was stuffed in Nojax casings and treated for 15 minutes with hickory smoke. Cooking was then carried out in steps by maintaining the cooking chamber temperature for 15 minutes at each of the following temperatures in ascending order: 60°C, 65.6°C, 71.1°C, 76.7°C and 85°C. Internal temperatures of the product were measured to ascertain that a temperature of 68.6°C was reached. After cooking the products were washed with a spray of water, dried in air and stored

in a cooler at 2°C until they were used for organoleptic evaluation.

Methods of evaluation:

Analyses of fish muscles and FPC were conducted in duplicate on samples randomly drawn from the homogenous mass of each batch or several batches pooled together. Fish muscles requiring dehydration prior to analyses were freeze-dried at 5°C for 55 hours in an Industrial Dynamics Pilot Plant Freeze Dryer Model, CPF-20.

(a) Chemical analyses

1. Protein determinations were based on Kjeldahl nitrogen content x 6.25. Initial determinations were made by the AOAC (1965) method. However, sulfuric acid digestion with a mixture of selenium oxide and potassium sulfate as catalysts (Willard et al., 1956) was found to give higher and more reproducible values than the AOAC procedure. Soluble protein in supernatants was estimated by the biuret procedure of Snow (1950).

2. Moisture and/or volatile substances were determined by heating to a constant weight in a drying oven at 100°C (AOAC, 1965).

3. Fat content was determined by extraction with diethyl ether in a soxhlet for 8 hours (AOAC, 1965).

4. Ash content was determined by ashing in a muffle furnace at 550°C (AOAC, 1965).

5. Amino acids were analyzed in a fully automated Beckman 120 Model C Amino Acid Analyzer. Samples were saturated with nitrogen and then hydrolyzed with 6N HCl under vacuum at 110°C (Stein and

Moore, 1954). Tryptophan was determined by the alkaline hyrolysis procedure of Kohler et al. (1967). Cysteine was analyzed by the performic acid oxidation method as described by Hirs (1967).

6. Cd and As were determined according to the AOAC (1970) procedure. Trace amounts of Pb were determined by the procedure described by Hoover et al. (1969). Hg was measured by flameless absorption spectrometry (Hoover et al., 1971).

(b) Microbiological examination

The detection of salmonella, staphylococci, clostridia and the enumeration of coliform, aerobic and anaerobic viable organisms were according to the "Recommended Methods for the Microbiological Examination of Foods" (APHA, 1967). Presence of putrefactive anaerobes and Clostridium botulinum was further determined on the basis of the recommendations of the National Communicable Disease Center, Atlanta, Georgia, U. S. A., (USPHS, 1968).

(c) Determination of protein yield

The yield of FPC was determined as percent protein recovered. Kjeldahl nitrogen values were used and periodically checked with true protein values calculated from amino acid analyses. The loss of protein during processing was computed by difference or by determining the amount of protein lost in the solvents.

(d) Titration curve

A titration curve was developed by adding 0.0197N NaOH solution to the FPC solution (2 gm/100 ml) and measuring the corresponding change in pH.

(e) Short-term feeding experiments

Ten rats of the Sprague-Dawley strain, each weighing about 60 g were chosen for feeding studies. Five of the rats were fed the control diet containing 10 percent vitamin-free casein. The other five rats were fed the test diet containing 10 percent FPC. The remaining 90 percent of both diets was identical. It was prepared according to the nutritionally balanced protein-free basal diet formulation of Breuer et al., (1966). In the test diet $NaHCO_3$ was added to adjust the pH of FPC to 7.0. An equivalent amount of NaCl was added to the control diet. The rats were fed ad libitum for 4 weeks. At the end of the third week 0.13 percent methionine was added to the control diet to remove any methionine deficiency. Daily weight gain and feed intake of each rat were used to calculate the protein efficiency ratio (PER) as described by Munro and Allison, (1964).

The rats were sacrificed at the end of four weeks for gross and histopathological examination. Abnormalities in the organs, lesions in the tissues and any apparent differences between the rats fed on the control and test diets were recorded. Samples of blood, kidney and liver were collected from the rats for the analysis of mercury.

(f) Rehydration capacity of FPC

Estimates of water holding capacity of FPC were made by rehydrating it in water at different pH and ionic strengths. Samples of FPC, 0.2 gram each, were rehydrated with 10 ml of distilled water in a 50 ml centrifuge tube for at least one hour at room temperature

(25°C). The pH was adjusted with 1M NaOH or 1M HCl. The ionic strength was adjusted to the same value in each tube by adding NaCl and distilled water until the final volume of the rehydrating medium was 15 ml. The rehydrated FPC was then centrifuged at 2400 x G for 30 minutes. The supernatant was removed very carefully by capillary pipette and its volume was measured. The amount of water retained by the FPC was calculated by subtracting the volume of supernatant from the total volume of the rehydration medium (15 ml). Correction for the evaporation loss during centrifugation was made by using water blanks.

The rehydration capacities at pH 2 and 5 were estimated in a similar way at the ionic strengths of 0.3, 0.6, 0.9 and 1.2. The pH was adjusted with 1M NaOH. The solution was adjusted to the required ionic strength with NaCl.

(g) Emulsifying capacity estimation

The emulsifying experiments were conducted according to the scheme of Carpenter and Saffle (1964). Concentrations of FPC ranging from 1 to 2.5 gm/100 ml of water were emulsified in a blender by adding corn oil continuously at 12 ml/minute. The results were plotted as concentration of FPC in water against percent oil in the emulsion at the inversion point.

(h) Organoleptic evaluation

1. Randomly selected families in the Bryan-College Station area were used for product evaluation. A questionnaire was developed for a consumer evaluation (Figure 5) of the sausage

NAME _____

QUESTIONNAIRE # _____

TEXAS A&M SAUSAGE STUDY

Please score the sample of sausage for the various characteristics according to the following scoring system:

SCORE	DESCRIPTION
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like or dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

For each characteristic (Aroma, Chewiness, Smokiness, etc.), write in the space labeled "Comments" why you gave the score you did.

I. Preparation Score (Preparing the sausage)

	SCORE	COMMENTS
Ease of Preparation		
Aroma		
General Appearance		
Overall Satisfaction		

II. Palatability Score (Eating the sausage)

	SCORE	COMMENTS
Aroma		
Juiciness		
Grittiness		
Chewiness		
Spiciness		
Smokiness		
Saltiness		
Overall Satisfaction		

Fig. 5 - Questionnaire for organoleptic evaluation by consumers.

from fresh fish which had been considered acceptable in a preliminary taste panel evaluation. Factors for evaluation were chosen on the basis of preliminary investigations. A hedonic scale from 1 to 9 was used for extreme dislike to extreme like of the factor under evaluation (Peryam and Pilgrim, 1957).

2. A questionaire was developed for the evaluation of the FPC substituted product (Figure 6) by a randomly selected group forming an untrained taste panel. A hedonic scale of 1 to 8 was used showing extreme dislike to extreme like. The provision for the score of 5 showing neither like nor dislike was eliminated in this study to force the members of the taste panel to make a decision for either like to dislike. Factors to be evaluated were chosen on the basis of a preliminary evaluation of the product. A control sample without FPC was used to compare the differences between the test samples and the control samples.

Number of experimental batches of FPC prepared for its evaluation and characterization:

Although numerous batches of FPC were prepared during the course of study, three batches of fish muscle and FPC made from the fish muscle were extensively analyzed. Short term feeding experiments were conducted on five control and five test rats with a pooled sample of FPC obtained from the three batches. In the sausage formulations for the organoleptic evaluation, a pooled sample of 750 gm of FPC was prepared from 15 batches of fish with low mercury

NAME _____

QUESTIONNAIRE # _____

TEXAS A&M SAUSAGE STUDY

Please score the sample of sausage for the various characteristics according to the following scoring system:

SCORE	DESCRIPTION
8	Like extremely
7	Like very much
6	Like moderately
5	Like slightly
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

I. a) Palatability Score (eating the sausage):

SCORE

	Sausage No.	Sausage No.	Sausage No.
Color			
Flavor			
Mouthfeel			
Overall Satisfaction			

b) Any other comment (write in) _____

_____

II. Now, RANK - 1, 2, and 3 your preference of these sausages:

1. Sausage # _____
2. Sausage # _____
3. Sausage # _____

Fig. 6 - Questionnaire for organoleptic evaluation by a taste panel.

content (<0.05 ppm). Qualitative tests for rehydration and emulsification were conducted on each batch of FPC. Quantitative tests for rehydration and emulsification capacities were conducted at 2.5 on several batches. The values reported are from the pooled sample of FPC used for sausage formulation. All analyses were performed in duplicate unless otherwise mentioned. Duplicate results which varied more than 10 percent were repeated.

CHAPTER V

RESULTS

Process development:

Based on the theoretical discussion presented in Chapter III a process for the preparation of FPC was developed. Ethanol was chosen as the primary solvent for the removal of lipids and odoriferous compounds at pH 2.5. The use of ethanol as the main solvent was considered to provide two advantages over the commonly used solvent isopropanol:

1. Ethanol is not toxic at low levels, thus eliminating the necessity of complete removal of solvent residues.

2. It is less reactive with acids than isopropanol (Noller, 1958).

Table 2 presents the data on the extraction of protein from fish muscle at pH 2.5 with ethanol and different concentrations of NaCl. Gel formation was observed when the NaCl concentration was less than 3 percent of the weight of fish muscle. The protein residue or pellet obtained after centrifugation tended to be more granular as the NaCl concentration increased. At the 5 percent level of NaCl separation of solvent by filtration was the easiest as the protein residue was granular and the supernatant clear. Turbidity in the supernatants persisted from 0 to 4 percent NaCl. The material responsible for the turbidity appeared to clog filters

Table 2 - Extraction of fish muscle (100 gm) at pH 2.5

NaCl (gm)	Ethanol (ml)	Nature of protein	Volume of supernatant (ml)	Appearance of[a] supernatant
0	600	Gel	68-488	Turbid
1	600	Gel	333-516	Turbid
2	600	Gel and granular	448-502	Turbid
3	600	Gel and granular	514-647	Turbid
4	600	Granular	492-510	Turbid
5	600	Granular	503-526	Clear

[a]Total protein lost in supernatant ranged from 0.3-2.7 mg/100 gm based on Kjeldahl nitrogen determination.

thus making solvent removal difficult. The volume of supernatant recovered was indicative of the volume of the gel which varied considerably with the lower salt concentrations. The reason for the variation in gel formation has not been established. However, there is some evidence that increased water content might cause increased gel formation.

Protein recoveries were determined at pH 2, 2.5, 3, 4 and 7 (Table 3). Recovery values at pH 3.0 were highly variable but are reported in Table 3. Ethanol and 5 percent NaCl were used as the extracting medium. The highest protein recovery was 61 percent at pH 2.5 and the lowest was 48 percent at pH 7. Recoveries were even lower for high fat fish (10 percent fat). This appeared to be caused by (1) a detergent effect of the lipid and (2) the need for additional extraction steps for lipid removal. Therefore, use of a secondary solvent was considered. Hexane, an immiscible organic solvent which has been considered as a suitable solvent for the preparation of FPC by several workers (Pariser and Odland, 1963; Yanez et al., 1967), was chosen to increase the rate of extraction of neutral lipids. The addition of hexane to the system increased protein recovery, when the frozen fish were used, from 61 percent to an average value of 86 percent (Table 3). Recovery values were 2 - 10 percent higher for fresh fish than frozen fish.

The state of subdivision of the fish muscle was highly important. If the material was not finely ground, the larger particles appeared to trap lipid and upon drying would not rehydrate.

Table 3 - Protein recovery under different systems of solvent and pH

Fish (g)	NaCl (g)	EtOH (ml)	Hexane (ml)	pH	Protein recovery[a] (%)
100	5	200	-	2.0	54
100	5	200	-	2.5	61
100	5	200	-	3.0	50
100	5	200	-	4.0	49
100	5	200	-	7.0	48
100	5	200	200	2.5	86[b]

[a]Recovery values are listed for frozen fish as percent of original protein content of fish muscle.

[b]Recovery values for fresh fish were 2 - 10 percent higher.

Consequently, this resulted in the development of fishy odors which could be removed by sieving out the larger particles. Efficient stirring was necessary during pH adjustment and subsequent extraction to remove lipid and prevent odor reversion.

If ethanol was added prior to pH adjustment it took approximately 30 minutes before the pH became stable at 2.5. Therefore, it was necessary to titrate one or two samples and to add this volume of acid (about 19 ml of 1N HCl/100 gm of fish muscle) to the subsequent samples. The pH adjustment is more rapid if the HCl is added prior to the addition of ethanol. However, very rigorous stirring or mixing is necessary to achieve uniform pH adjustment.

The lipids were not studied in detail. In the binary solvent system the ethanol - water layer contained protein while the hexane layer on top contained most of the lipid. The color of the lipid ranged from light straw color, when fresh fish were used, to an orange or red color when frozen fish were used. The orange or red color was indicative of oxidized lipids while a straw color indicated a high quality lipid (Tsuchiya, 1961). Exposure to light and oxygen for a few days caused polymerization of the lipids. Adjustment of the ethanol extract to pH 9.0 with $Ca(OH)_2$ produced a precipitate with strong fishy odor and orange color, even when the fresh fish were used. This precipitate appeared to remove most of the lipids (presumably phospholipids) from the ethanol.

The FPC prepared from fresh fish by ethanol-hexane extraction with 5 percent NaCl at pH 2.5 had the following properties:

1. A dry free flowing powder (Figure 7), off white in color [the color appears to depend upon species; FPC prepared from Brevoortia Spp. (menhaden) and Mugil cephalus (mullet) was grey]

2. No detectable odor

3. Hydrophilic but not hydroscopic

4. Slightly sour taste prior to pH adjustment, but after the pH was adjusted above 5, no taste

5. Could be stored for 6 months or more in unsealed containers at room temperature without any development of unpleasant odors.

When dried to a constant weight at 105°C the dry powder contained about 97 percent protein. However, to maintain functional properties the FPC was usually dried to a constant weight at 70°C. The ratio between the weight at 70°C and the weight at 105°C provided a simple means for estimating protein recoveries.

Some fish from Galveston Bay which had evidently been caught from polluted waters had tainted flesh with petroleum and other obnoxious odors. These odors were sometimes difficult to remove from the protein. Also, some of the hexane was found to impart petroleum odors during processing. This was due to an impurity in the hexane and could be diminished by distilling the hexane and discarding the first and last 15 percent of distillate. Other sources of off odor were from the viscera when whole fish were used. When feedy fish had been stored on ice or frozen in the round, odor removal was more difficult than from fish which had been eviscerated while still fresh.

Fig. 7 - Dry fish protein concentrate.

Gross composition of fish muscle and FPC:

The proximate analyses of fish muscle and FPC are presented in Table 4. Extensive analyses were performed on three batches of fish muscle and FPC prepared from them. The percent protein in fish muscle ranged from 17.88 to 18.80 whereas it was 86.18 to 87.74 in the FPC. The fat percent in fish muscle was 0.42 - 0.59. The residual level of lipid was reduced to 0.31 - 0.36 percent in the FPC. Although this was slightly higher than the suggested levels to prevent odor reversion (Dambergs, 1969), no odor reversion was noticed even after prolonged storage (6 months) of the FPC at room temperature (25°C). The volatile content in fish muscle was 79.82 - 81.11 percent which was decreased to 9.06 - 10.36 percent in FPC. The ash content was 0.59 - 0.82 percent in fish muscle and 2.75 - 3.51 percent in FPC. Since boneless fillets were used in this study, the ash levels were considerably lower than those expected when whole fish are used. There was no evidence that the NaCl used in the processing was substantially increasing the ash level.

Amino acid analyses of fish muscle and FPC:

The amino acid composition of fish muscle and FPC made from the muscle are presented in Table 5. Average values of duplicate assays are reported for each batch. Although batch to batch variation was observed, the average values indicated that, except for lysine, the fish muscle and FPC had similar essential amino acid spectra (Figure 8). Tryptophan, which might be destroyed by the acid extraction

Table 4 - Proximate analysis of fish muscle and fish protein concentrate

Analysis[a]	Fish muscle			Fish protein concentrate		
	Batch I	Batch II	Batch III	Batch I[b]	Batch II	Batch III
Protein	18.80	18.77	17.88	86.18	87.74	87.08
Fat	0.45	0.59	0.42	0.36	0.31	0.35
Volatile	80.02	79.82	81.11	10.36	9.20	9.06
Ash	0.73	0.82	0.59	3.10	2.75	3.51

[a]Expressed as percentage.

[b]Batch numbers of FPC correspond to batch numbers of fish muscle from which the FPC was made.

Table 5 - Amino acid analysis of fish muscle and fish protein concentrate

Analysis[a]	Fish muscle[b]			Fish protein concentrate		
	Batch I	Batch II	Batch III	Batch I[c]	Batch II	Batch III
Lysine	10.70	10.89	10.68	9.70	10.25	9.71
Histidine	2.18	2.17	2.20	2.05	2.17	2.09
Arginine	6.93	6.88	6.82	6.84	6.90	7.75
Aspartic Acid	10.50	10.75	10.56	10.19	10.49	10.08
Threonine	4.52	4.34	4.36	4.45	4.48	4.32
Serine	3.31	3.10	3.09	3.26	3.30	3.19
Glutamic Acid	16.45	16.91	16.01	15.51	15.75	15.15
Proline	3.62	3.17	3.18	3.86	3.49	4.19
Glycine	3.87	3.85	3.76	5.07	3.64	5.11
Alanine	5.82	5.87	5.76	6.05	5.67	5.97
Cysteine[d]	0.76	0.85	0.96	0.92	1.22	0.99
Valine	5.60	5.55	5.88	5.30	5.52	5.45
Methionine	3.63	3.70	3.83	3.71	3.80	3.69
Isoleucine	5.28	5.30	5.37	5.36	5.37	4.99
Leucine	8.51	8.63	8.65	8.32	8.54	7.95
Tyrosine	3.97	2.52	3.31	3.83	4.15	3.85

Table 5 - Amino acid analysis of fish muscle and fish protein concentrate (continued)

Analysis[a]	Fish muscle[b]			Fish protein concentrate		
	Batch I	Batch II	Batch III	Batch I[c]	Batch II	Batch III
Phenylalanine	4.54	4.38	4.49	4.27	4.50	4.34
Tryptophan[e]	1.08	1.20	1.12	1.41	1.14	1.23

[a]Amino acid concentration in gm/100 gm of protein.

[b]Fish muscles were freeze dried prior to analysis.

[c]Batch numbers of FPC correspond to batch numbers of fish muscle from which the FPC was made.

[d]Determined as cysteic acid.

[e]Determined by basic hydrolysis.

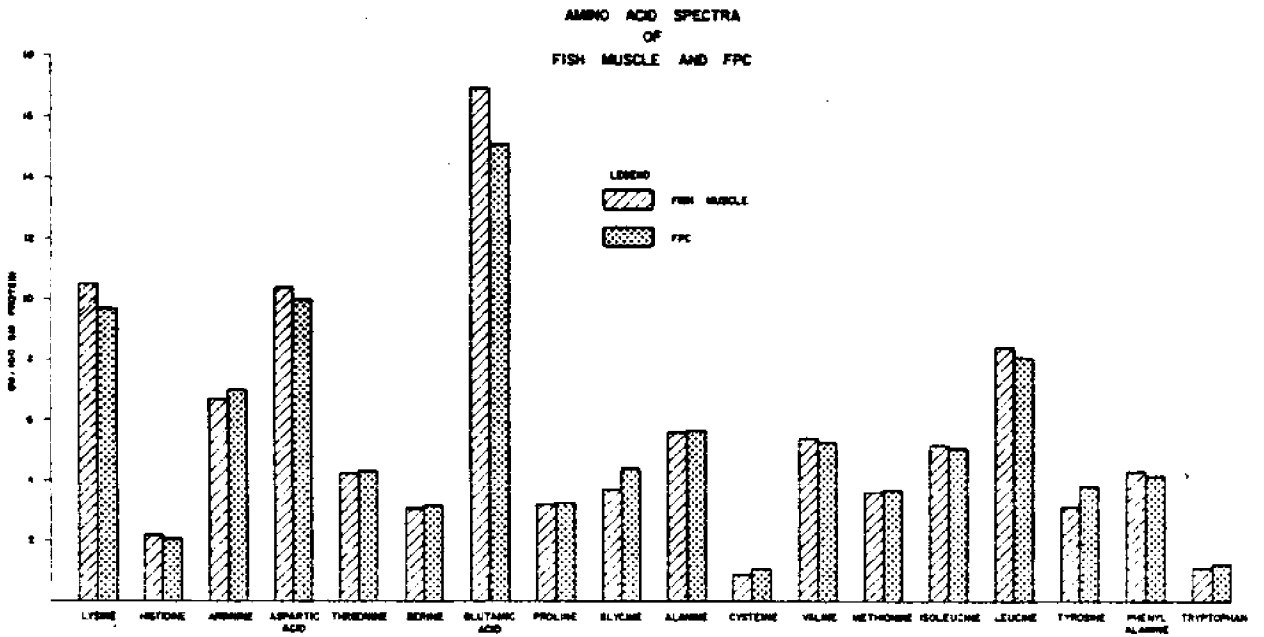


Fig. 8 - Comparative histogram of amino acid content of fish muscle and fish protein concentrate.

conditions, was preserved. From the analysis of the hydrolysates, it appeared that some cysteine might be lost during the process. However, cysteine assayed by performic acid oxidation did not show any decrease indicating that the difference was due to destruction of the cysteine during hydrolysis. Nutritionally, the FPC had an excellent amino acid balance which was consistent with the reports of BCF (1966) and Dubrow et al., (1970).

Bacteriological examination of fish muscle and FPC:

Duplicate samples of the three batches of fish muscle were examined for microbial flora prior to processing into FPC. Immediately after preparation of FPC duplicate samples from each batch were removed aseptically and examined for the change in the bacterial flora. The results are presented in Table 6. Anaerobic agar plate counts per gram of fish muscle were higher than the aerobic plate counts in Batch I and III, and in Batch II they were almost equal. This suggested that there were very few strict anaerobes which was confirmed by further incubation of the anaerobes under aerobic conditions. Processing of the muscle into FPC destroyed most of the microorganisms. No viable organism was observed in any of the different batches except in one sample of Batch I. This probably was due to contamination during analysis since the duplicate sample did not produce growth, and no growth was evident in enrichment media (tripticase-soy agar). No growth in the lowest dilution was reported as less than 5 per gram. Further examination of fish muscle

Table 6 - Bacteriological examination of fish muscle and fish protein concentrate

Sample	Batch[a] No.	Agar plate count/gm		Coliform MPN/0.1gm	Coagulase positive staphylococci	Salmonella sp.	Anaerobic sporeformers
		Aerobic	Anaerobic				
Fish muscle	I	2.2×10^4	7.1×10^4	3.6	-[b]	+[c]	-[b]
	I	7.0×10^4	8.6×10^4	<1.8	-	--[d]	-
	II	1.1×10^5	8.9×10^4	<1.8	-	--	-
	II	1.0×10^5	9.8×10^4	<1.8	-	--	-
	III	5.6×10^4	6.0×10^4	<1.8	-	--	-
	III	5.4×10^4	1.0×10^5	3.6	-	--	-
Fish protein concentrate	I	<5	<5	<1.8	-	--	-
	I	<5	25	<1.8	-	--	-
	II	<5	<5	<1.8	-	--	-
	II	<5	<5	<1.8	-	--	-
	III	<5	<5	<1.8	-	--	-
	III	<5	<5	<1.8	-	--	-

[a]Batch numbers of fish protein concentrate (FPC) correspond to batch numbers of fish muscle from which the FPC was made.

[b]Represents <1 microorganism per gram.

[c]Represents at least 4 microorganisms per gram.

[d]Represents <4 microorganisms per gram.

revealed coliform bacteria (3.6 MPN/0.1 gm) in one sample each of Batch I and III. These were reduced to 0 in the FPC. No coagulase positive staphylococci and anaerobic sporeformers were found in the fish muscle and FPC. No Clostridium botulinum was detected. Batch I was salmonella positive for one sample of the fish muscle. However, all the samples of FPC were salmonella negative. In general, the process appeared to yield sterile FPC. This was not unexpected since solvent and pH conditions were not conducive to bacterial survival.

Analyses of hazardous metals in fish muscle and FPC:

Petroleum and other obnoxious odors in the fish obtained from Galveston Bay suggested that they were from polluted waters. Heavy metals such as Pb, Cd, As and Hg which were reported to be present in Galveston Bay as pollutants (Copeland and Fruh, 1970) were analyzed to determine their levels in the fish muscles. FPC samples were also analyzed for the heavy metals to observe the effect of processing. Table 7 presents the levels of Pb, Cd and As in the three batches of muscle and the corresponding samples of FPC. The Pb content (0.4 ppm) in fish muscle of Batch I was reduced during processing to <0.2 ppm in FPC. In Batch II the Pb level was too low for detection in either the muscle or FPC. However, in Batch III a slight concentration from <0.2 ppm in the muscle to 0.4 ppm in the FPC was observed. Cd was less than 0.1 ppm in all of the fish muscle and FPC. The As level which varied from 4.4 to 4.8 ppm in fish muscle was removed during processing, thus reducing the level

Table 7 - Analysis of Lead, Cadmium and Arsenic in fish muscle and fish protein concentrate (FPC)

Batch[c] No.	Sample	Analysis[a] Pb	Cd	As
I	Fish muscle[b]	0.4	<0.1	4.8
II	Fish muscle[b]	<0.2	<0.1	4.4
III	Fish muscle[b]	<0.2	<0.1	4.4
I	FPC	<0.2	<0.1	<1.0
II	FPC	<0.2	<0.1	<1.0
III	FPC	0.4	<0.1	<1.0

[a]Concentration in ppm.

[b]Freeze dried prior to analysis.

[c]Batch numbers of fish protein concentrate (FPC) correspond to batch numbers of fish muscle from which the FPC was made.

in FPC to less than 1 ppm. The residual levels of Pb, Cd and As were so low in the FPC that they could not be considered as hazardous (Underwood, 1971).

Analyses of Hg in fish muscle and FPC are presented in Table 8. Hg levels in the three batches of fish muscle were 0.304, 0.189 and 0.243 ppm. These were concentrated in FPC to 1.713, 1.147 and 1.380 ppm, respectively. The concentrations were higher in the fish muscle than the values of 0.02 to 0.18 ppm reported by Underwood (1971) for Hg in "uncontaminated" fish. Almost all the Hg in fish muscle was recovered in FPC. The recovery appeared to be proportional to the protein yield.

Short term feeding study:

The high level of Hg in the fish muscle, which could not be removed during processing, suggested that feeding studies with rats should be conducted to observe possible toxicity of the FPC. Another purpose of the study was to observe the growth and calculate the protein efficiency ratio (PER) which is a measure of the nutritional value of the protein. Prior to the preparation of the FPC diet, a titration curve (Figure 9) was developed so that $NaHCO_3$ could be added to neutralize the acidity of the FPC. This was necessary because rats cannot tolerate acidity (Breuer et al., 1964). The neutralization reaction would produce NaCl in the FPC diet (test diet). To compensate this an equivalent amount of NaCl was included in the casein diet (control diet).

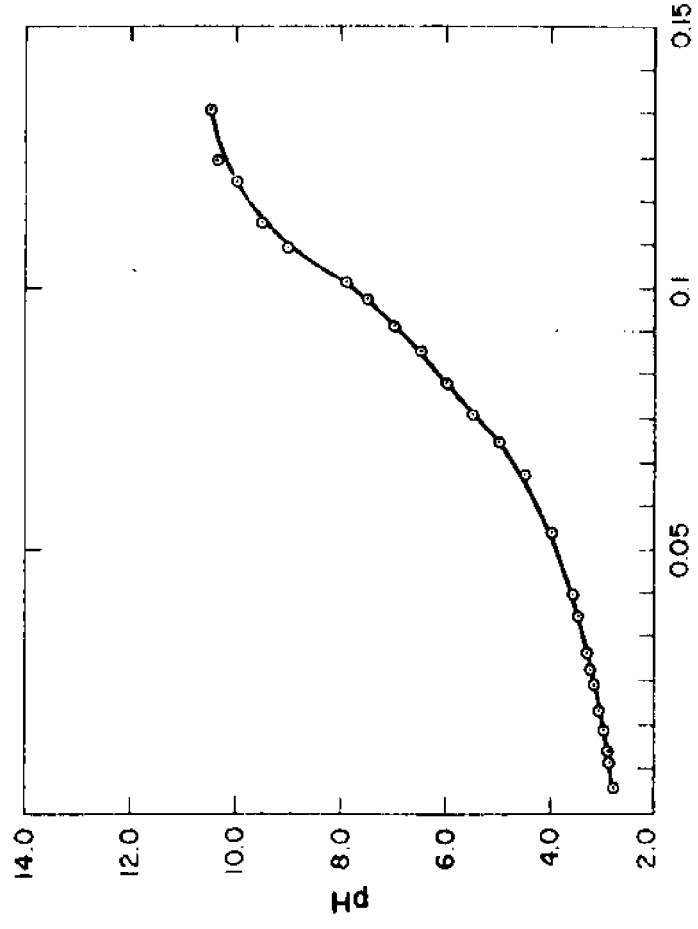


Fig. 9 - Titration curve for fish protein concentrate.

Table 8 - Analysis of Mercury in fish muscle and its concentration in FPC

Batch No.	Hg in wet fish muscle (ppm)	Hg in FPC[a] (ppm)	FPC produced[b]	Protein yield[c]	Hg recovered[d]
I	0.304	1.713	17.20	74.16	96.90
II	0.189	1.147	16.80	82.15	98.10
III	0.243	1.380	21.03	99.69	119.10

[a]Average values of three determinations.

[b]Per 100 gm of wet fish muscle.

[c]Protein in FPC as percent of protein in fish muscle prior to processing.

[d]Hg in FPC as percent of Hg in fish muscle prior to processing.

The rats grew better on the FPC diet than the control diet. Table 9 presents the growth data. PER values ranging from 1.042 to 2.586 were observed for the rats on control diet while the PER values for the FPC-fed rats varied from 2.895 to 3.699. The latter were significantly higher ($P<0.01$) as determined by the pooled "t" test (Snedecor and Cochran, 1967).

At the conclusion of the experiments, the rats were sacrificed and examined for histopathological abnormalities by a veterinarian. Except for roughness of the skin coating in the control rats and a respiratory infection in both control and test rats, no abnormality was found.

Analysis of Hg in the control diet showed the level to be below 0.03 ppm, while in the test diet made by using a pooled sample of FPC, the level was 0.07 ppm. The Hg contents of the blood, livers and kidneys of control and test rats are presented in Table 10. In the blood of the control rats the Hg level varied from 0.07 to 0.14 ppm while in the blood of the test rats it varied from 0.36 to 0.52 ppm. In the livers of the control rats the Hg level was <0.05; whereas it was 0.45 to 0.51 ppm in the livers of the test rats. The Hg levels in the kidneys of the control rats varied from <0.05 to 0.2 ppm, but in the kidneys of the test rats the levels ranged from 0.64 to 0.98 ppm.

Table 11 presents the Hg intake of the test rats and the percent retained by the kidneys, livers and blood. The total retention of Hg in the kidneys, livers and blood ranged from 27.7 to 44.79

Table 9 - Growth data of rats[a] for 4 weeks

Rat No.	Diet	Initial[b] wt. grams	Final[c] wt. grams	Av. daily wt. gain grams/day	Wt. of[d] kidneys grams	Wt. of[d] liver grams	Av. daily protein consumption grams/day	PER
1	Control	59.5	154.0	2.946	1.215	7.380	1.128	2.613
2	"	53.3	95.0	1.132	0.855	4.980	0.816	1.388
3	"	48.7	89.0	1.046	0.700	5.296	1.004	1.042
4	"	55.5	124.0	2.036	1.010	6.275	0.811	2.509
5	"	55.3	136.0	2.464	0.954	6.310	0.956	2.586
6	Test[e]	58.2	171.0	3.707	1.201	7.699	1.281	2.895
7	"	53.7	152.0	3.707	1.125	7.395	1.195	3.103
8	"	53.7	179.5	4.357	1.398	7.630	1.202	3.699
9	"	60.5	173.0	4.018	1.255	7.064	1.141	3.520
10	"	50.8	159.5	3.829	1.129	6.998	1.227	3.121

[a]Sprague-Dawly strain of male rats were fed ad libitum for 4 weeks.

[b]Weight of rats prior to the commencement of feeding trial.

[c]Weight on the 29th day prior to the necropsy of the rats.

[d]Weight just after necropsy.

[e]PER values for test diet significantly higher than control ($P < .01$).

Table 10 - Analysis of Mercury on rat blood, liver and kidney

Rat No.	Diet description of rats	Mercury (ppm)		
		Blood	Liver	Kidney
1	Casein (control)	0.085	<0.05[a]	0.20
2	"	0.140	<0.05	0.12
3	"	0.070	<0.05	<0.05
4	"	0.130	<0.05	0.18
5	"	0.080	<0.05	0.10
6	FPC (test)	0.390	0.51	0.89
7	"	0.470	0.45	0.97
8	"	0.520	0.51	0.98
9	"	0.360	0.49	0.64
10	"	0.370	0.50	0.72

[a]Below the level of detection of analysis.

Table 11 - Mercury retained in the kidney, liver and blood

Rat No.	Mercury intake[a] (µg)	Mercury retained[b]			
		Kidney	Liver	Blood	Total
6	25.929	4.12	15.14	15.74	35.00
7	24.386	4.47	13.64	18.13	36.24
8	24.424	5.60	15.93	23.26	44.79
9	23.263	3.45	14.87	16.38	34.70
10	28.699	2.83	12.19	12.68	27.70

[a]Total intake of Hg through feed consumption.

[b]Hg retained as percent of total intake.

percent of the Hg intake. Hg retained in the kidneys was 2.83 - 5.60 percent of intake; in the livers, 12.19 - 15.93 percent; and the blood, 12.68 - 23.26 percent. The values for the blood were calculated on the basis of data for the blood volumes of rats complied by Miller (1969). The accumulation of Hg in the test rats was above suggested hazardous levels (Anon., 1969).

Functional properties of FPC:

(a) Rehydration capacity

The rehydration properties of FPC depend upon pH and ionic strength. Figure 10 shows the water holding capacity of pooled samples of FPC at low ionic strength (0.133) under different conditions of pH. The lowest water holding capacity was in the region of pH 5 - 8. The water holding capacity decreased from 16 to 7 ml per gram as the pH increased from 2 to 7. As the pH was increased from 7 to 10 the water holding capacity increased from 7 to 20 ml per gram. In the pH zone of 5 to 8, which covers the pH of most of the sausage emulsions, the water holding capacity varied from 10 to 7 ml per gram. This was approximately twice the amount of the original water in the fish muscle (70 to 80 percent depending upon the fat content). Rehydration of FPC to the approximate original water content of fish muscle (20 percent FPC, 80 percent water) between pH 5 and 8 yielded gels having the consistency of a thick paste (Figure 11). The water holding capacity was markedly increased when the ionic strength was increased from 0.133 to 0.4 at pH 5 and

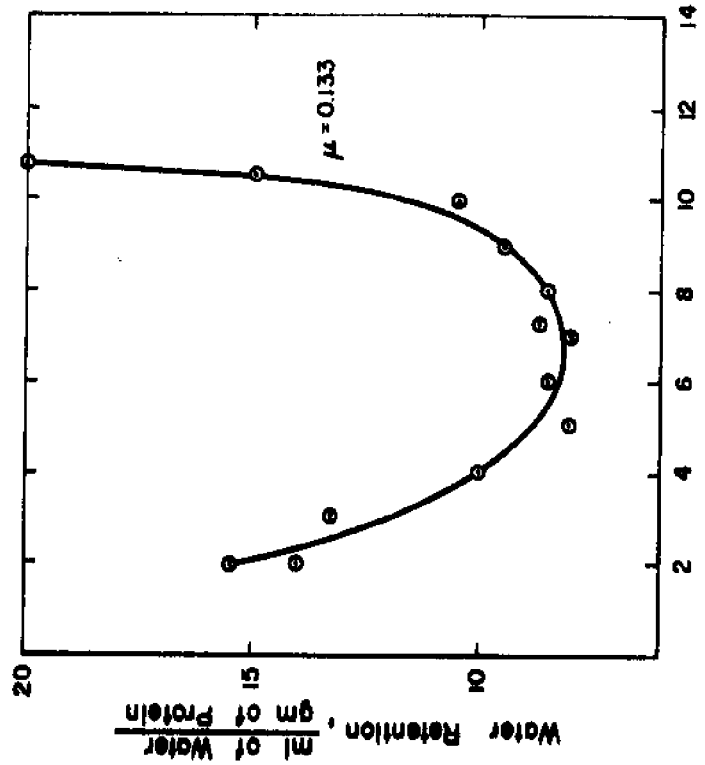


Fig. 10 - Water holding capacity of FPC at different pH.

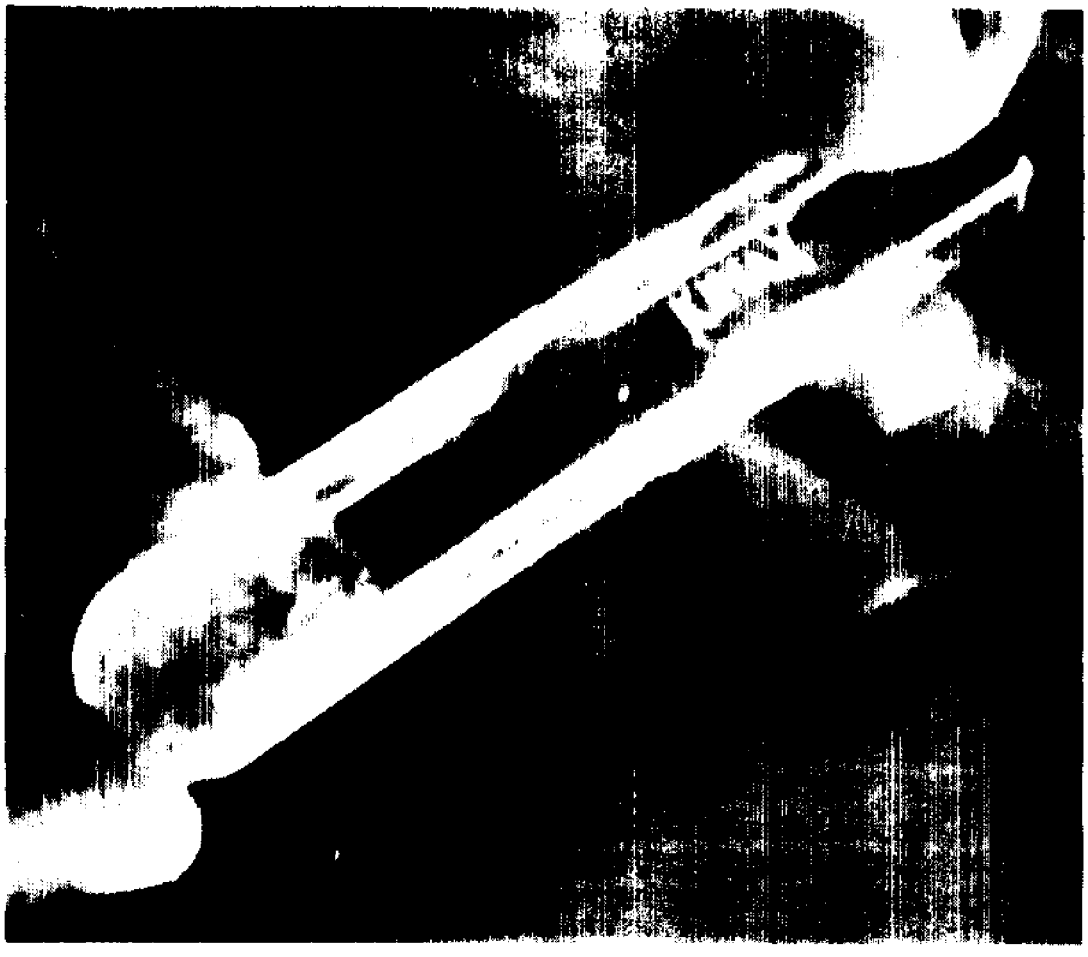


Fig. 1 -

then there was a gradual decrease with further increase of the ionic strength to 1.2 (Figure 12). At pH 2 there was no change in the water holding capacity with the change of the ionic strength from 0.133 to 0.4. Then the water holding capacity decreased as the ionic strength was increased to 0.9, after which it appeared to increase. The water holding capacity may be affected by heat, as some rehydrated samples appeared to loose a small amount of water upon heating to 100°C in a boiling water bath. This was evident with only a few samples and did not occur if the sample had been emulsified with Wesson oil prior to heating.

Change of pH and salt content had a marked effect on the FPC which had been fully rehydrated at acid pH. The expulsion of water was observed when pH was changed from 2 to 5. An increase in the water holding capacity at pH 5 was evident when salt was added to the rehydrated FPC.

The pH of FPC prior to drying was found to be critical for its rehydration capacity. If the pH was adjusted to 4 while the FPC was wet, the dried FPC did not rehydrate. Adjustment of pH was done by employing NaOH, Na_2CO_3, $NaHCO_3$, $CaHPO_4$ and in the case of small amount of FPC, the addition of beef protein was sufficient to change the pH of the paste.

(b) Emulsifying capacity

The emulsifying capacity of FPC was studied by rehydrating the pooled samples of FPC in water and blending it with Wesson oil to the inversion point at which the emulsion could no longer hold any

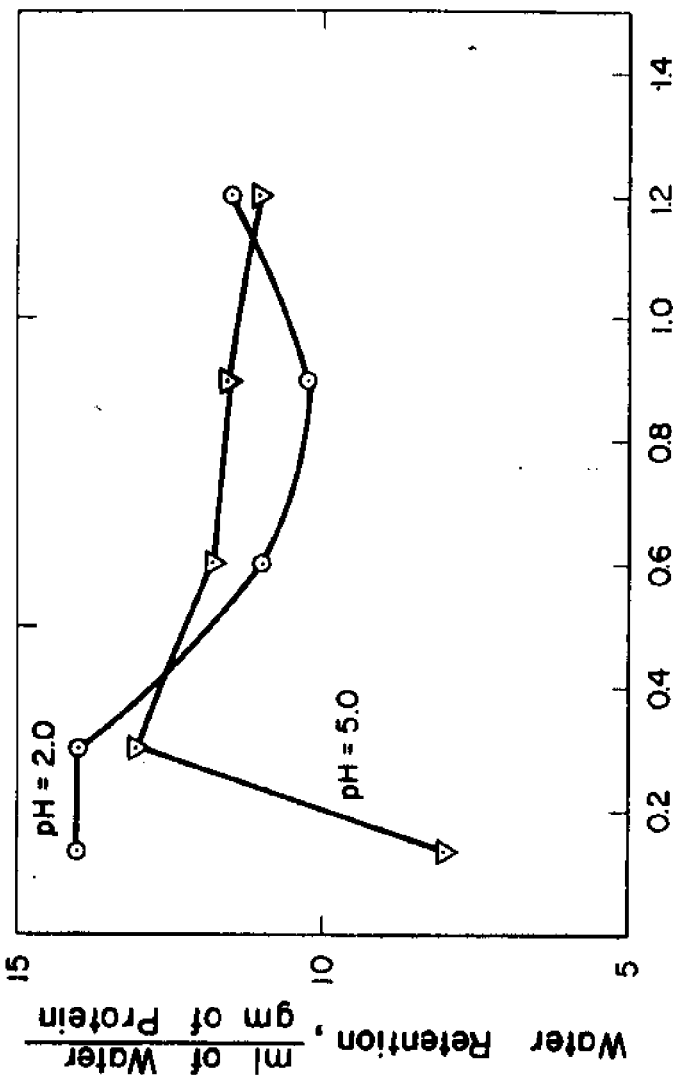


Fig. 12 - Water holding capacity of FPC at different ionic strength.

more oil. The inversion point was difficult to observe when low concentrations of FPC (below 0.75 gram of FPC in 100 ml of water) were used. Figure 13 shows the variation in the percent of oil with different concentrations of FPC in emulsions at the inversion point. At pH 3 the maximum oil content in the emulsion was nearly 60 percent by volume when the FPC concentration of 1 gm/100 ml was used. The emulsion was thick, creamy and smooth (Figure 14).

A study of the emulsifying properties at different pH was conducted with a concentration of FPC of 1 gm/100 ml of water. The percent of oil in the emulsion varied from a maximum of 60 percent at pH 3 to a minimum of 40 percent at pH 5 (Figure 15). When the pH of the saturated emulsions made at pH 2 or 3 was changed to a more basic pH, a loss of oil and water was evident. If the emulsion was not fully saturated with oil, tasteless milky suspensions were often formed when the pH was adjusted to the region of 7. Temperatures up to 100°C did not appear to affect the emulsions.

(c) Protein solubility

Protein solubility at the different values of ionic strength and pH listed in Tables 10 and 12 was too low for measurement.

Evaluation of sausage-like products:

The development of sausage-like products from comminuted M. undulatus was complicated by (1) grittiness due to the presence of bone fragments and scales, (2) undesirable odors in fish and (3) oxidation of lipid. A mild heat treatment, 90°C for 30 minutes,

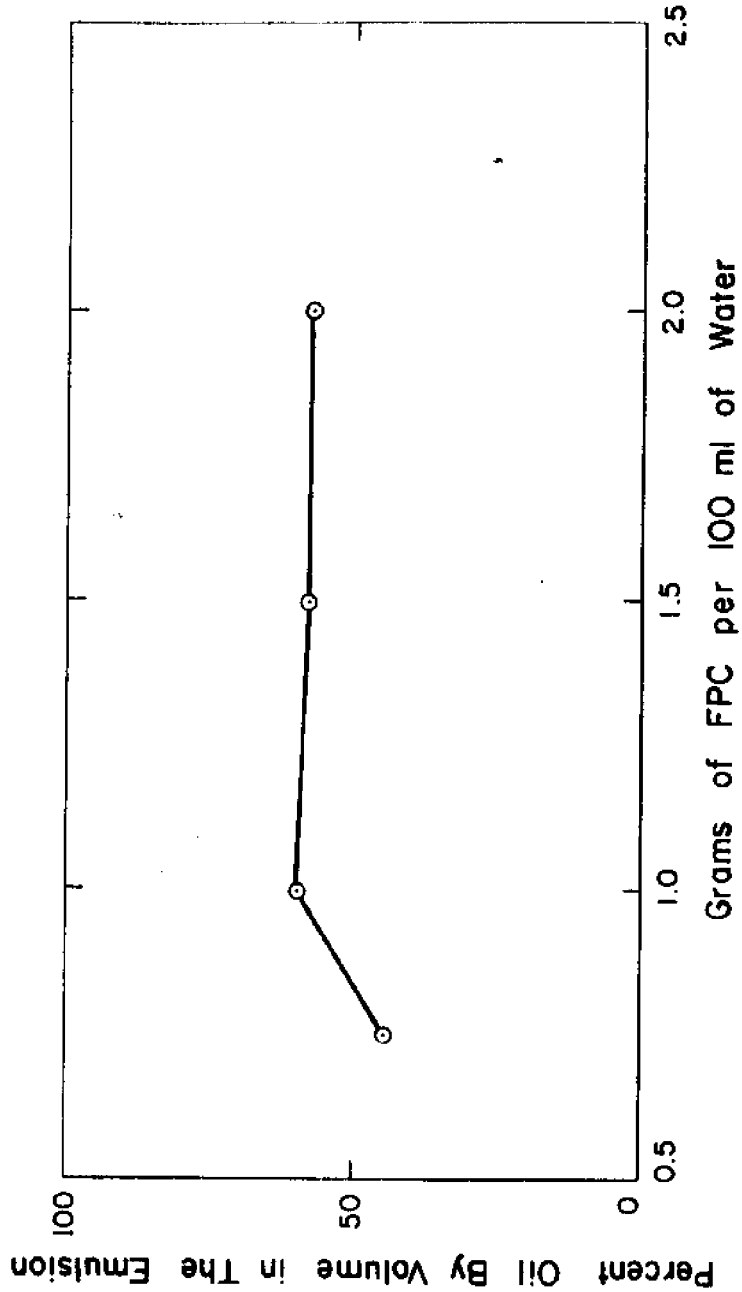


Fig. 13 - Emulsifying capacity of different concentrations of FPC at pH 3.0.

14 - Fish protein concentrate - corn oil

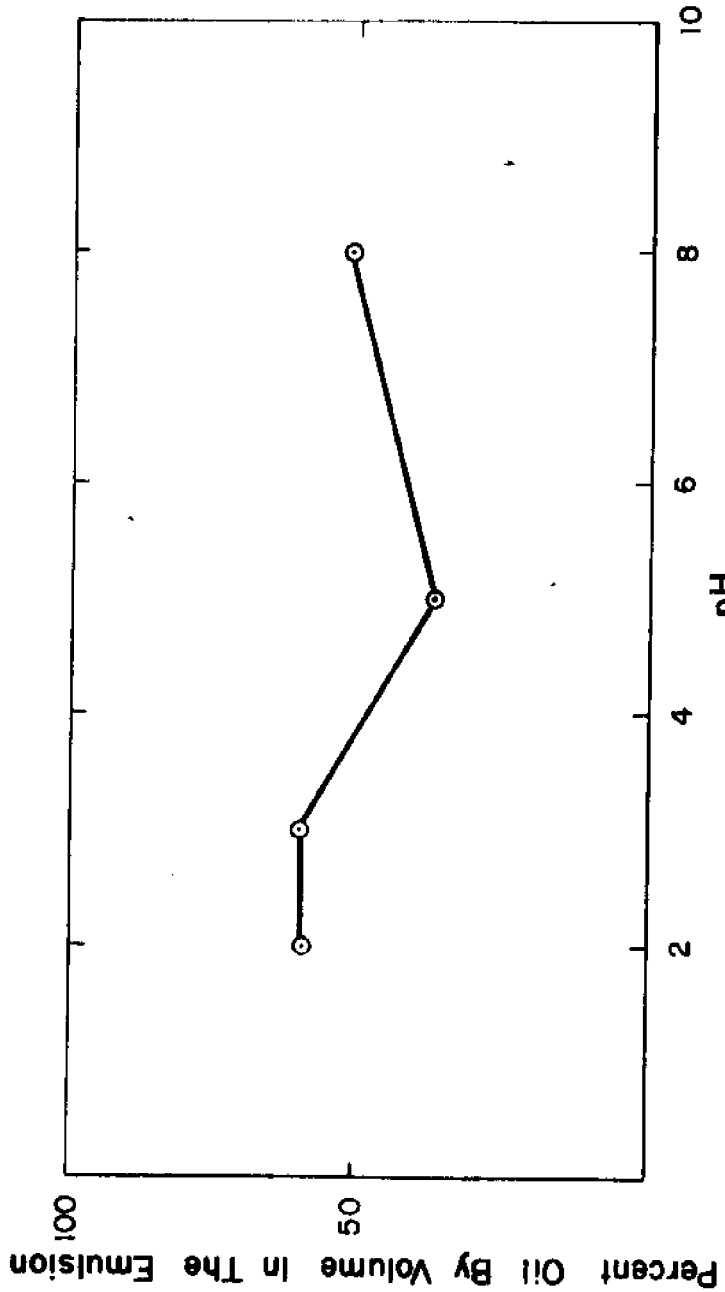


Fig. 15 - Emulsifying capacity of FPC at different pH.

softened the bone fragments. However, any remaining scales were hardened by the process. Complete removal of the scales was necessary, but often difficult to accomplish. In many of the batches of fish, particularly those from Galveston Bay, undesirable odors could not be masked. Lipid oxidation was controlled by the addition of saturated fat (Lovern, 1966) and liquid smoke (Watts and Faulkner, 1954). The latter also helped mask undesirable odors.

One batch of fish, free from off-odors, was used to prepare a large quantity of sausage-like product for organoleptic evaluation by 185 judges. The sausage was considered to be suitable for human consumption by preliminary testing by laboratory personnel. During the cooking process, browning and shrinkage of the product were encountered. This caused a less than desirable appearance of the product and probably affected subsequent evaluation. In the organoleptic evaluation the palatability scores for overall satisfaction showed 34.6 percent of the judges considered the product acceptable, 9.2 percent neither liked nor disliked the product and 56.2 percent expressed unacceptability. From the overall satisfaction scores profile, it appeared that the product was rated as unsuitable because of its flavor.

A large amount of FPC was prepared from a batch of M. undulatus which had a low Hg content (<0.05 ppm) and no petroleum odor. Wiener type products ir which FPC was substituted for beef were prepared. During preliminary investigations, several batches of the product were prepared by substituting beef with rehydrated FPC up to

the 20 percent level. The products appeared to be acceptable for color and texture during preliminary taste panel tests in the laboratory. However, their flavor was considered to be different from the usual wieners. No unpleasant or fishy odors were noticed. Spiciness, saltiness and a slight toughness were evident. These were attributed to dehydration of the sausages during smoking and cooking operations.

For organoleptic evaluation, three batches of sausage-like products of the wiener type formulation were prepared: (1) control without any FPC, (2) FPC I, 20 percent FPC substituted and (3) FPC II, 40 percent FPC substituted. During preparation, the smokehouse controls did not work satisfactorily. Consequently, the sausages may have been overcooked. Figure 16 shows the appearance and texture of the sausages. The control and FPC I appeared to be close in texture and appearance (Figure 16 A,B). FPC II (Figure 16 C) was shrivelled and had a coarse texture as seen in the cross and transverse sections.

The proximate analyses of the sausages (Table 12) did not show much variation between the control and FPC I (2 percent variation in protein and moisture content). However, dehydration in the FPC II was evidenced by a low moisture content, 51 percent against 56.6 percent for the control and 58.8 percent for FPC I. In FPC II the drop in moisture content raised the protein, fat and ash levels to 24.9, 20.0 and 4.1 percent, respectively, compared to 20.4, 19.2 and 3.8 percent for protein, fat and ash levels in the control

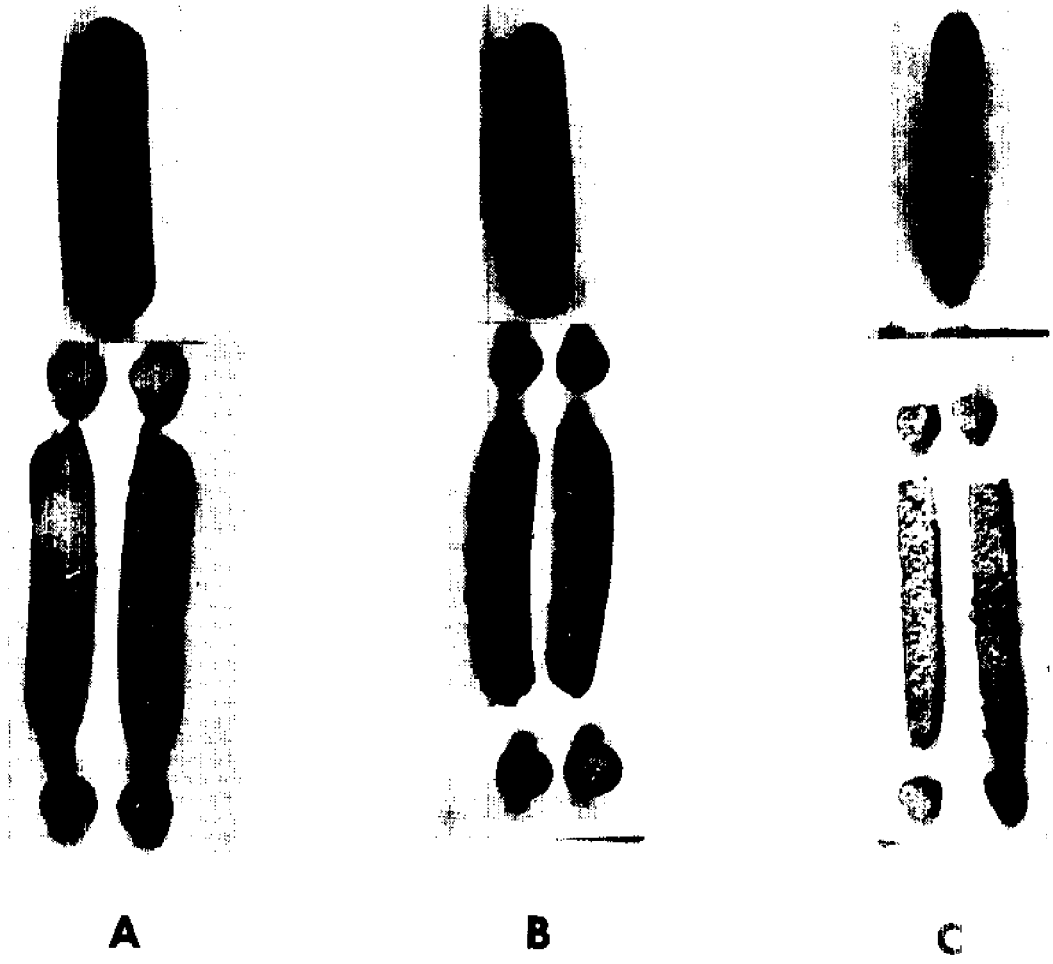


Fig. 16 - Control and fish protein concentrate products (A) control, (B) 20 percent beef replaced with rehydrated FPC, (C) 40 percent beef replaced with rehydrated FPC.

Table 12 - Proximate analyses of sausages[a]

Analysis[b]	Sausages		
	Control	FPC I[c]	FPC II[d]
Protein[e]	20.4	18.1	24.9
Fat	19.2	19.6	20.0
Moisture	56.6	58.8	51.0
Ash	3.8	3.5	4.1

[a]The sausages were analyzed after being smoked, cooked and stored for 48 hours at 2°C.

[b]Expressed as percentage.

[c]20 percent beef substituted with rehydrated FPC

[d]40 percent beef substituted with rehydrated FPC

[e]Determined by difference (<5 percent variation from Kjeldahl nitrogen values).

sausage. This indicated a lesser water holding capacity of the formulation of FPC II under the conditions of heating, smoking and storage.

The microbial quality of the control, FPC I and FPC II sausages were determined by the agar plate count method (APHA, 1967). The aerobic counts per gram of the product ranged from 4.1×10^3 - 9.6×10^3, and the anaerobic counts per gram varied from 5.7×10^3 to 1.1×10^4. The bacterial load of the control did not show any big difference from that of the sausages made from FPC (Table 13).

Table 14 shows the percent of judges of a 34 member taste panel who had considered the products acceptable (scored 5 and above in the 8 point hedonic scale). The control sausage was rated acceptable by all the judges for flavor and overall satisfaction. For color, 88.3 percent liked it; and, for mouthfeel, 91.2 percent showed acceptability. FPC I was acceptable to 20.6 percent for flavor and overall satisfaction, 64.7 percent for color and 8.8 percent for mouthfeel. FPC II was rated as acceptable for flavor and overall satisfaction by 5.9 percent of the judges and for color by 50 percent of the judges. No one expressed liking for the mouthfeel because of its poor texture and grittiness. Appearance and color seemed to influence the percent of likes and dislikes. The overall satisfaction scores reflected the preference of flavor.

Table 15 presents the mean scores of the organoleptic evaluation by the 34 member taste panel. The overall satisfaction scores were 6.2 for the control, 3.4 for FPC I and 2.6 for FPC II in the 8 point

Table 13 - Microbial quality of sausages

Sausage	Agar plate count per gram of sausage	
	Aerobic	Anaerobic
Control	7.2×10^3	6.2×10^3
FPC I[a]	4.1×10^3	5.7×10^3
FPC II[b]	9.6×10^3	1.1×10^4

[a]20 percent of beef substituted with rehydrated FPC.

[b]40 percent of beef substituted with rehydrated FPC.

Table 14 - Frequency of scores expressing acceptability of the sausages[a]

Sausage	Percent of judges rated the product acceptable[b]			
	Color	Flavor	Mouthfeel	Overall satisfaction
Control	88.3	100	91.2	100
FPC I[c]	64.7	20.6	8.8	20.6
FPC II[d]	50.0	5.9	0	5.9

[a]Acceptable score was 5 and above in an 8 point hedonic scale.

[b]Total number of judges on the taste panel was 34.

[c]20 percent beef substituted with rehydrated FPC.

[d]40 percent beef substituted with rehydrated FPC.

Table 15 - Mean score of the organoleptic evaluation of sausages[a]

Sausage	Score for the attributes evaluated[b]							
	Color		Flavor		Mouthfeel		Overall satisfaction	
	Mean	Stand. dev.	Mean	Stand. dev.	Mean	Stand. dev.	Mean	Stand. dev.
Control[c]	5.7	0.89	6.3	0.91	6.0	0.88	6.2	0.79
FPC I[d]	4.9	1.84	3.5	1.40	3.0	1.26	3.4	1.34
FPC II[e]	4.6	1.44	2.8	1.40	2.1	1.00	2.6	1.29

[a]Mean scores based on 8 point hedonic scale (extreme like = 8, extreme dislike = 1) judged by a 34 member taste panel, each sausage judged twice.

[b]Mean scores for color, flavor, mouthfeel and overall satisfaction were significantly different for the three sausages at $P<0.05$.

[c]Control sausage contained 50 percent beef and 50 percent pork in a wiener type emulsion.

[d]20 percent of the beef replaced by rehydrated FPC.

[e]40 percent of the beef replaced by rehydrated FPC.

hedonic scale. Color, flavor and mouthfeel scores all indicated the same trend, the higher the FPC content, the lower the score. The mean score in each category for the control and FPC substituted sausages were significantly different from each other ($P<0.05$). FPC I was considered to be close to acceptability. Although no fish flavor was detected by any judge in either FPC I or FPC II, it was evident that improvement in formulation was needed to make the flavor and mouthfeel more desirable. The adverse comments by the judges concerning the FPC substituted sausages were: tasted like Vienna sausage, too salty, too spicy and dry.

CHAPTER VI

DISCUSSION

Fish protein concentrate (FPC) is one of the recognized means of utilization of "trash fish" for human consumption. However, the absence of rehydration and emulsifying capacities of FPC limits its use to that of a nutritional food supplement. Devanney and Mahnken (1970) in a review of the economics of FPC have discussed this aspect as follows:

> It has also been suggested that FPC might find a substantial market in the processed meat field such as sausages and meat patties. In this regard, it is interesting to note that the sausage industry distinguishes between functional protein and filler protein. Functional protein is required to have texture, binding power, and taste, and presently is comprised of meats for which the industry pays 40 cents per pound of protein and more. Filler protein is expected to supply little more than bulk. Currently, the industry uses by-products associated with the packing industry which are available at about 3 cents per pound or about 10 cents per pound of protein. Presently, FPC cannot compete with functional protein on the basis of its properties nor with filler protein on the basis of its costs (Smith, 1968). If, however, FPC's functional properties, particularly with respect to binding power, which natural fish protein has, could be improved, it would open up a tremendous market.

The development of a procedure to maintain functional properties in FPC appears to be necessary to make the process economical (Holden, 1971). Such a process has been developed and described in the preceding chapters of this dissertation.

A number of problems were encountered during the development of

a process to utilize Micropogon undulatus. It soon became evident that suitable fish for a comminuted product were not available in sufficient quantity for continued research. Undesirable odors and flavors prevented utilization of fish from Galveston Bay. The supply of fish from the Gulf of Mexico was complicated by two factors: (1) the shrimpers, even when offered a good price, refused to land a small quantity of fish and (2) the fish were too small to be utilized commercially in a comminuted product. Some of the techniques employed during this research such as the prevention of oxidation of lipid by addition of saturated fat are being explored for possible commercial utilization in fish cakes.

Preliminary studies provided ample evidence that the removal of undesirable odors was necessary for product development work with M. undulatus. Extraction of protein at 5°C and 25°C with 5 percent NaCl and precipitation at pH 2.5 did not produce odorless protein pellets. Subsequent washing of the pellets at 5°C with water, 3 percent NaCl solution, 3 percent NaCl solution at pH 2.5, 95 percent ethanol and 95 percent ethanol at pH 2.5 did not remove undesirable odors. Chu and Pigott (1970) noted a similar odor problem in their process of HCl-water extraction of fish protein. As a result, they suggested that their product be used for animal feed. Stillings and Knobl (1971) also reported odor problems in aqueous, enzymatic and solvent extracted fish protein concentrates processed at room temperature. These processes evidently did not produce FPC with low levels of residual lipids which were stable against odor reversion.

Consequently, solvent extraction processes at room temperature were considered as unsuitable for making an acceptable material for food usages. Therefore, a hot solvent procedure was indicated.

Although the hot solvent extraction process which was developed appeared to overcome some of the difficulties of a comminuted product, the initial quality of the starting material was important. The presence of petroleum and mercury made some batches of fish unsuitable as starting material. Also, feedy fish which had not been eviscerated possessed odors which were difficult to remove. This suggested that it may be important to start the process soon after the fish are caught. The yields of protein and lipid decreased considerably when fish which had been frozen were used as starting material. This appeared to be the result of lipid (probably oxidized) - protein interaction. Such lipid protein - interactions have been credited with reducing protein extractability (Anderson et al., 1965). Oxidation during frozen storage, as judged by color, reduced the quality of the oil. Since the fish used in this study were preserved in a manner not feasible for commercial operations, this suggested that fresh fish should be used for the starting material.

Health authorities will not allow "spoiled" fish to be used in the preparation of FPC (USFR, 1967). Consequently, the preservation of the fish prior to processing is very important. Such methods as refrigerated brine may be suitable. An ethanol-salt mixture at pH

2.5 appears to preserve the fish without refrigeration for several days. The possible use of this material for preservation of fish aboard shrimp boats has been discussed (Cobb and Hyder, 1971). There are several advantages of preserving the starting material with what might be regarded as an initial stage in the extraction process: (1) refrigeration costs are eliminated, (2) the risk of rapid spoilage is reduced, (3) labor costs would probably be lowered because handling should be reduced and (4) processing costs should be lowered because the lipid-rich material floats to the top where it could be skimmed off.

Knobl (1967) has discussed the qualities of a solvent for extraction of raw fish or fish meal. According to these criteria the binary solvent of ethanol-hexane provides the following advantages: (1) the efficient removal of water, polar and non-polar lipids and odor-bearing compounds without dissolving or reacting with proteinaceous components, (2) the low boiling points of hexane and ethanol allow for inexpensive desolventization of the concentrate and the avoidance of heating to excessive temperatures, (3) the solvents can be recovered by simple distillation, (4) the solvents can be deodorized by distillation and treatment with activated charcoal, (5) the solvents are inert toward metallic equipment although the low pH of 2.5 would make the slurry corrosive and (6) ethanol and hexane are produced commercially for use in the food industry.

The solvent system has the following disadvantages:

(1) Hexane is highly flammable, (2) the cost of hexane and ethanol, depending upon the country in which the process is to be employed, may be more expensive than other proposed solvent systems and (3) if breathed, hexane can be toxic, particularly to plant employees.

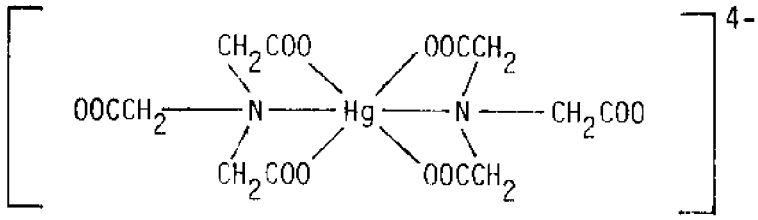
Variations in the solvent process need to be studied more extensively. For instance, with low fat content fish it may be possible to eliminate the use of hexane after the first extraction. Replacement of ethanol by isopropanol may be desirable, particularly if the solvent residue level in the FPC can be reduced by improved drying methods or steam stripping while maintaining functional properties of the protein. Humidification and redrying, as has been used to reduce solvent residues in isopropanol - extracted FPC (Ciprios et al., 1968; Finch, 1970), might allow isopropanol to be used in the process. The use of isopropanol, however, will also depend upon the degree of reaction with acid in the solvent medium. The presence of heavy metals such as zinc would be expected to increase the rate of reaction of alcohol and acid (Noller, 1958).

Lovern (1966) has emphasized the importance of oil recovery by pointing out the fact that "it is the oily pelagic fish that can be caught most cheaply in greatest quantities." The economics of FPC manufacture from such oily fish makes oil recovery a very important factor. Little attention has so far been given to the commercial production of oil as a co-product of FPC production. Recent concern

for offsetting the cost of FPC production has generated interest in this area. However, only one commercial enterprise (Astra, 1969) has undertaken the study of oil recovery on a large scale. The processing of fish oils is similar to that of vegetable oils but the lack of uniformity makes fish oil processing more difficult. The processing of fish oils for edible products usually consists of degumming, refining, bleaching, hydrogenation and deodorization (Chang, 1967). Hexane-ethanol extraction provides in the hexane an oil which has been degummed and hence partially refined. This is because phospholipids are extracted into the polar solvent, ethanol, while neutral fat is extracted into the nonpolar hexane.

A comprehensive study of solvent and by-product recovery is required to determine (1) amount of solvents lost per unit of FPC produced, (2) removal of undesirable odors from solvents during recovery, (3) whether co-current or countercurrent extraction should be used and (4) the amount and quality of nitrogenous compounds which are obtained as by-products of solvent recovery and may be used as an animal feed supplement.

The presence of Hg in the FPC prepared from fish taken from polluted waters suggested that Hg was tightly bound in a covalent linkage to the sulfur amino acids as (1) - S - Hg - S and/or (2) - S - Hg - CH_3 (Dunlap, 1971). Under the processing condition of low pH, if Hg had been in a chelated form as indicated for triglycine:



(Johnson and Callis, 1956), it should have been removed. The lack of a charge on the carboxyl group and positive charges on the - NH_2 and other basic groups would have precluded chelate formation.

Although no histopathological abnormalities were evident after 4 weeks of feeding, the rapid incorporation of Hg from FPC into the kidneys, livers and blood of FPC fed rats suggested a possible health hazard. The hazards of low levels of Hg ingestion over a long period of time are not yet known. Before FPC from Hg contaminated fish can be used in human foods, long term feeding studies are necessary. Feeding trials should be conducted in different species of animals.

These studies indicate that Hg in fish meal has a distinct possibility of being transmitted into poultry and other meat producing animals which are fed on the meal. In future studies special attention should be given to (1) accumulation of Hg in brain tissues and its effect on the nervous system, (2) histopathological abnormalities in the organs such as kidneys, livers and heart due to incorporation of Hg into them and (3) the effects of Hg on reproduction.

The possible health hazards of alkyl mercury (Anon., 1969) require lowering of the Hg level in FPC made from contaminated fish.

Perfection of this step would make possible utilization of the fish which have been barred from the market due to Hg contamination.

Finch (1970) states that the proteins may be used in foods for their functional properties rather than their nutritive properties. He adds that FPC may be made by processes which in order to secure desired physico-chemical properties reduce protein quality. One such commercial process was the Wiking Eiweiss process in Germany (Shenstone, 1952) which produced an egg white substitute from fish protein. The product had good whipping properties but the manufacturing conditions of solubilization with hot alkali suggest that the protein was degraded. In contrast, the process developed in this study provides not only a protein with rehydration and emulsifying properties but it also preserves most of the essential amino acids originally present in the fish muscle. The slight loss of lysine that occurs during processing suggests that lysine rich proteins, possibly histones or peptides, are lost. This material may be recovered in the solvent residue, making it more valuable as an animal feed supplement.

Mattil (1971) discussing the functionality of proteins has emphasized that a protein should be soluble under the conditions of pH, ionic strength, etc., at which the protein will be used. This study has however, indicated that factors such as rehydration and emulsification may be more important than solubility. Although the FPC was insoluble at ionic strength 0.133 - 1.2 and pH 5 - 8, its water holding and emulsifying capacities made it suitable for use

in emulsified products such as sausages.

Incorporation of the FPC at low levels (20 percent beef substituted with rehydrated FPC) in sausage-like products indicated that it possessed some binding power. Preliminary studies have also showed that: (1) a milk-type product could be prepared by emulsifying the FPC (0.5 gm/100 ml of water) with corn oil below the inversion point and (2) patties could be produced by mixing FPC (5 parts), wheat flour (1 part), water (3 parts) and vegetable oil (1 part). All of the studies indicated that the FPC could be utilized in developing a family of high protein products such as beverages, patties, sausages, etc. However, further studies are required to determine: (1) the optimum concentration of FPC in emulsified products, (2) the methods of treating FPC such as partial rehydration, emulsification, solubilization, adjustment of pH and ionic strength prior to its incorporation into the products, (3) the conditions of smoking, curing and cooking of the products and (4) the effect of various ingredients such as flours, spices, salts, fats and oils on flavor, color and texture of FPC products.

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