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Preparation of Fish Protein Concentrate (FPC) from Shark

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PREPARATION OF FISH PROTEIN CONCENTRATE (FPC) FROM SHARK

By

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ABSTRACT

The meat of sharks and other members of the family Elasmobranchii contains urea. Urease, present in fish muscle or released by microbes grown during storage, catalyzes the decomposition of urea to ammonia, which is responsible for off-flavor and odor in products from fish containing urea. Therefore, it is necessary to remove urea in the preparation of FPC (fish protein concentrate) from shark meat, if the FPC is to be utilized as a highly nutritive animal protein supplement in food to combat human malnutrition in developing and underdeveloped countries.

For the production of urea-free FPC, multistage cross-current solvent extraction, multistage countercurrent solvent extraction and an aqueous phosphate process in combination with multistage countercurrent solvent extraction were tried. Ethanol (95%) and commercial hexane were used as solvents to remove urea, moisture and lipids in the crosscurrent and countercurrent methods. In the aqueous phosphate process, hexametaphosphate at pH 4.0 was used to precipitate protein from an aqueous medium while urea was removed with the water phase. Subsequent solvent extraction reduced the moisture and lipid levels to below the FDA limits of 10% and 0.5%, respectively.

In all stages the solvent was added in the ratio of one ml per gram of ground raw fish. Starting with shark meat containing 1.1% urea and 13.4% lipids, the urea and lipid levels in the FPC were 0.16 and 0.61%, respectively, following countercurrent solvent extraction

using three ethanol stages followed by three hexane stages with 15 minute extraction periods. Six crosscurrent ethanol extractions with 60 minute extraction periods reduced the urea level in the FPC to a trace, while the lipid was 0.41%. The FPC produced by these methods was white or light cream colored with no or slight amine and fishy odor and good functional properties.

The FPC produced by the aqueous phosphate process followed by countercurrent solvent extraction was better in color, odor, flavor and texture than FPC produced by the solvent extraction alone. The urea and lipid levels in the FPC were 0.07 and 0.56%, respectively.

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I. INTRODUCTION

Fish protein concentrate (FPC) is defined in various ways. One of these is "Fish protein concentrate is any low cost, stable, wholesome product of high nutritive quality, hygienically prepared from fish, in which the protein and other nutrient materials are more concentrated than they were in the fresh fish" (Stillings and Knobl, 1971). The characteristics of the products may range from tasteless, odorless, light-colored, flour-like materials to colored products having a slightly fishy taste and odor. This concentrated protein can be used as a supplement in human diets to combat protein malnutrition (Moorjani and Lahiry, 1970).

The population of the world is, perhaps, growing at a faster rate than the production of foods for them. Over and again, the protein foods are already in alarming deficiency in the underdeveloped and developing countries and even among some groups of people in the developed countries.

In most of the developed and developing countries, fishes like anchovies (Linson, 1966), cod (Fougere, 1962 and Pariser, 1963), haddock (Guttmann and Vandenheuvel, 1957), hake (Caiozzi et al., 1968), menhaden (Whaley and Moshy, 1965) and sardine (Brown and Miller, 1969) are used in the production of FPC. Very little attention is given to a fairly large family of fish, the Elasmobranchii. The Elasmobranchii is comprised of sharks, skates and rays. Among the smaller varieties of sharks, some are commonly known as dogfish.

Three-quarters of the surface of the earth is covered with salt water. The vast area of tropical and sub-tropical salt waters like the Caribbean, the Gulf of Mexico, the Bay of Bengal, the Arabian Sea, the south coast of Australia and New Zealand and the west coast of the U.S.A. are heavily infested with sharks and other members of this family (Borgstrom, 1962). It has been reported that about 33 percent of the total catch of fish in the Bay of Bengal is Elasmobranchii (Hussain, 1967) and 25 percent in the Arabian Sea off the coast of Karachi, Pakistan is shark (Mahdihassan, 1962). It has also been reported that as much as 30 percent of the total catch in the Gulf of Mexico and the Caribbean (Bullis, 1957) and about 20 percent in the Indian Ocean (Kataoka, 1958) is damaged by sharks.

The utilization of shark meat for FPC production may serve two distinct purposes: (1) provide high protein food for human and animal nutrition and (2) reduce the damage to the other valuable fish by sharks, thereby boosting the yield of protein food for the people. In addition, the production of protein food may be increased by feeding urea-free FPC from Elasmobranchii meat to monogastric animals such as poultry and swine (Osterhaug, 1961). For these purposes, sharks may be harvested judiciously to maintain the balance in the natural ecology and also to get maximum production of shark meat for protein foods.

Shark meat contains a relatively high amount of urea (Alverson and Stansby, 1963). The latter compound decomposes to ammonia and carbon dioxide by the action of urease, an enzyme present in Elasmobranchii

muscle and can also be released by bacteria growing on the meat (Simidu and Oisi, 1952). Trimethylamine oxide (TMAO) is also present in Elasmobranchii meat (Groninger, 1959). This compound is reduced by enzymatic action in the fish muscle and by the microorganisms to trimethylamine (TMA). TMA has a very putrid fishy odor. Both ammonia and TMA evolved in the above-mentioned processes render off-flavor and taste to shark meat and its products, particularly when the products are kept in storage for a considerable period at room temperature (25 - 30 ^{o}C). So removal of urea, TMAO and TMA is important.

The lipids present in shark meat are also responsible for off-flavors on account of development of rancidity. The high moisture content helps the microbial spoilage of fish. As such, the U.S. Food and Drug Administration has set maximum levels of lipids and moisture in FPC at 0.5% and 10%, respectively (Appendix A).

Processing of shark meat will help in preventing microbial spoilage and will minimize storage, handling and transportation costs. Thus, finding a suitable method for producing FPC by removing urea, lipids and moisture from shark meat is the objective of this investigation.

II. REVIEW OF LITERATURE

FPC from Teleostei

The preparation of protein concentrate from fish, a highly perishable but an excellent nutritive commodity, is not a new but a very old idea. To save the nutritious meat of fish from complete spoilage, drying, salting and drying, smoking and making fish paste are still in use in the underdeveloped countries of the world. All these processes involve concentration of protein from fish. And the people use these products in their foods.

In ancient times, dry fish was not only used in human nutrition but also as fertilizer, after grinding, for increasing the productivity of the soil. Dried and ground fish known as fish-meal was used and even now is used in poultry and pet nutrition.

In the late nineteenth century, Waage in Norway prepared FPC with a good keeping quality for human consumption in the name of fish-meal (Bakken, 1961). In the process, fish fillets were chopped and dried in a high velocity air stream at a temperature of 40 ^{o}-60 ^{o}C to a moisture content of 5-6 percent. The product was then finely ground and sifted.

In 1936, the Norwegian Fish Powder Corporation produced fish-meal for human consumption (Bakken, 1961). The raw materials, mainly muscle from haddock and cod, were first cooked with live steam injected at a temperature of about 60 ^{o}C. After cooking, the mass was pressed in order to remove the glue materials. The press cake was dried under reduced

pressure at a temperature of about 40°C. The dried cake was sifted to separate bones which were ground to powder and mixed with fish-meal.

In Iceland, in 1938, an attempt was made to prepare fish flour from skinned cod fillets under sanitary conditions. The fillets were dried in a steam-jacketed vacuum drier (Hannesson, 1962).

In 1937, attempts were made in South Africa to produce tasteless and odorless fish flour for enriching cereal products intended for human consumption (Dreosti, 1962).

Since the second world war much attention has been given in all scientifically developed and developing countries to the preparation of the FPC to meet the ever growing need of protein food, to cut the length of the food chain used in converting fish to animal protein consumable by humans (Roels, 1969) and particularly to combat the problem of protein malnutrition disease, kwashiorkor, in developing and underdeveloped countries (Srikantia and Gopalan, 1966).

For this purpose, various methods have been developed to prepare FPC for human consumption. These methods can be placed under three main divisions. These are: (A) physical methods, (B) solvent extraction methods and (C) hydrolysis of fish. The combination of any two of these methods or all of the three methods may increase the efficiency of the process.

A. Physical Methods On the basis of this method, FPC plants have been set up in Congo/Ruanda-Burundi, Ghana and Uganda (Roels, 1969). In those plants fresh fish is autoclaved, the cooked fish is pressed to

remove fish solubles and lipids, and the press cake is dried, ground and packed in suitable sizes.

B. Solvent Extraction Methods These methods are widely used in the developed and developing countries like Canada, Chile, France, Germany, India, Japan, Mexico, Pakistan, Panama, Peru, Scandinavian countries, South Africa, The United Kingdom, The United States of America and Uruguay. The application of physical processes such as raising the temperature, stirring, filtration, pressing, distillation, vacuum drying, etc., increases the efficiency of the process. Some of the processes developed for the manufacture of FPC in some of the above mentioned countries are reviewed below.

Canada Workers at the Halifax Technological Laboratory of the Fisheries Research Board of Canada developed a process of manufacturing FPC using isopropanol (IPA) as the solvent for extracting water and lipids from fish muscle. The process is known as the "Guttmann-Vandenheuvel-Gunnarsson" process. They used cod fillets, whole cod, eviscerated cod, cod trimmings, haddock and hake as raw materials (Fougere, 1962). The process is briefly described here. The heads were removed and discarded, and the back bones and adhering muscles were ground in a meat chopper to quarter of an inch size. Equal weights of water and ground material were well mixed, acidified to pH 5.4-5.5 with polyphosphoric acid, and the acidified slurry was heated and stirred for 30 minutes at 65.5 ^{o}C. The slurry was centrifuged in a basket centrifuge and washed with hot water until the effluent was clear. The slurry was mixed with

twice its weight of IPA (86%), heated to 82°C for fifteen minutes and vacuum filtered. After a second solvent extraction and filtration, the extracted material was dried in an oven dryer at 37.5°C for 24 hours. The resultant dry material was screened through 16 and 20 mesh screens to separate bones and skins from protein and each fraction was ground in a Rietz grinder using a 1/32 inch screen. The fish flour thus obtained was, for all practical purposes, white, tasteless and odorless. This method has been improved by Power (1962).

Chile Whole hake was washed, comminuted and mixed with isobutanol in the ratio of 1:3 ground fish:isobutanol (w/w) with constant stirring for 30 minutes at room temperature and then at a temperature of 89.2-91°C for 4 hours. The extracted fish was then subjected to vacuum filtration. The fish cake thus obtained was washed twice with alcohol and finally it was filtered again. The FPC was dried at 60-65°C under reduced pressure to about 3-4 percent moisture (Hevia et al., 1971).

Germany This process was developed during World War II. Whole fish was ground and treated with 0.5% acetic acid with continuous stirring. The slurry was pressed and the press cake was extracted with ethanol. Following ethanol extraction, the press cake was hydrolyzed with alkali and filtered. The protein solution was neutralized with acetic acid and spray dried (Roels, 1969).

India FPC was produced from whole fish or eviscerated fish using ethanol (absolute or 96%) as solvent. The extractions were carried out in

six to seven stages at the boiling point of ethanol for a period of 15-20 minutes in each stage. The products were uniform from batch to batch. They were fine, non-gritty powders with light color and bland flavor (Moorjani and Lahiry, 1970).

Peru A process has been developed in this country to produce FPC from hake. The fish is first dried to a maximum moisture content of 8% by heat. The semi-dehydrated fish is sealed in an extractor and a vacuum is created, which opens the pores and cells of the fish material and exposes it more freely to hexane vapor which is used as solvent. The vaporized solvent removes all but less than 1 percent of the fat. Because of the low fat content, further deodorization is not necessary. The product is sterilized in the extractor (Philips, 1969).

Sweden "Astra Nutrition" produces FPC from herring using IPA as solvent. Fish is cooked with constant heat and stirring. The cooked fish is pressed and the pressed cake is subjected to successive extraction with solvent to reduce the fat content to the desired level. This defatted material is then desolventized and ground to FPC (Bakken, 1962; Roels, 1969; Lawler, 1970).

The United Kingdom Cavanagh and Inman obtained British patent #1,009,338 for the preparation of FPC by extracting fish with solvent mixtures of acetone, ethyl acetate and ethanol (Roels, 1969).

United States of America Various organizations have developed different processes in this country of which the following are important:

(1) Viobin Process In this process the fish is comminuted and the comminuted fish is dehydrated by an azeotropic distillation of water with ethylenedichloride. The dehydrated fish is then treated with more solvent to remove the lipids. Finally it is extracted with alcohol to get colorless and odorless FPC after grinding (Levin, 1959). The Viobin Corporation at Monticello, Illinois, the Alpine Marine Protein Industries, Inc. at New Bedford, Massachusetts, and the Cape Flattery Co. at Seattle, Washington, use the "Viobin Process" to produce FPC in industrial scale.

(2) The Bureau of Commercial Fisheries Currently known as the National Marine Fisheries Service, the Bureau has developed two methods of preparation of FPC.

(a) IPA Process Essentially this was first developed in Canada (Guttmann-Vandenheuvel-Gunnarsson Process) and further developed in the U.S.A. This process utilizes a successive series of extractions with azeotropic IPA moving countercurrent to the product to simultaneously dehydrate and defat comminuted fish. Residual solvent is then removed by either vacuum or atmospheric drying (BCF, 1966). This process is utilized to manufacture FPC by Star-Kist, Aberdeen, Washington.

(b) Aqueous Phosphate Process (Spinelli and Koury, 1970; Spinelli et al., 1971). In this process, fish is comminuted by grinding or by a "Yanagiya flesh separator" which also skins and debones the fish. The comminuted fish is mixed with an equal part of water and sufficient acid (H_2SO_4) is added to lower the pH to 5.7. The mixture is then rapidly heated to 70 -80 ^{o}C to inactivate the proteolytic enzymes.

Immediately after heating, sodium hexametaphosphate (1% based on weight of wet fish) in 5% aqueous solution is added to the acidified fish-water mixture. The pH of the slurry is then lowered to about 3.8-4.0 with 1M H_2SO_4. The slurry is centrifuged, yielding an aqueous-oil phase and a complexed solid fraction. The solid fraction is twice suspended in equal parts of water to remove other non-protein water soluble materials. The solid is then extracted twice with azeotropic IPA to remove water and residual oils. This is a new process. No industry has yet been developed on the basis of this process.

C. Hydrolytic Methods (The hydrolytic process for the preparation of FPC.) Two types of hydrolysis have so far been tried. These are (1) chemical hydrolysis and (2) enzymatic hydrolysis.

(1) Chemical Hydrolysis In this process whole fish is hydrolyzed chemically. The hydrolyzed product is filtered through a filter press to separate undissolved bones, skin and scale from protein solution. The filtrate is concentrated to about 50% solid and spray dried (Roels, 1969).

(2) Enzymatic Hydrolysis The process is identical to the chemical hydrolysis process, except that a proteolytic enzyme or a suitable microorganism is used in place of chemicals and the pH, temperature and concentration of the reacting media are controlled to achieve maximum activity of the enzymes. Rohn and Haas Company in Philadelphia, Pennsylvania in the United States (Roels, 1969) and the Tokai Regional Fisheries Research Laboratory in Japan (Onishi and Higashi, 1968) have developed methods to produce FPC by using proteolytic enzymes.

FPC from Elasmobranchii

In most cases anchovies, cod, ling-cod, hake, herring, haddock, pollack, sardine, whiting and similar species of fish have been used to manufacture FPC. Hardly any attempt has been made to utilize the species under the family Elasmobranchii in the scientifically developed countries. However, some attention has been given in the developing countries like India, Mexico and Pakistan to the preparation of FPC from elasmobranchs.

India In this country, Revankar et al., (1965) prepared FPC from shark. The shark meat was ground, cooked and pressed. The press cake was soaked in 1.0% acetic acid overnight. The acidified slurry was then filtered and the cake was extracted with 95% ethanol to remove oil and water.

Mexico A process has been worked out to prepare FPC from shark meat. In this process, sharks are eviscerated, bled and washed thoroughly with water. The meat is cut in 1/4 inch cubes, extracted with IPA at the temperature of 20 -30 C for a period of 50 minutes and filtered. A second extraction is carried out for 90 minutes at 75 C followed by a third extraction at 75 C for 75 minutes (Du Solier MacGregor and Cavazo, 1969).

Pakistan Abdul Haq (1960) prepared FPC from shark meat. To remove urea, minced shark meat was cooked with soy bean meal at 40 C. The mixture was dried at 50 -55 C to 10% moisture content, powdered, and extracted

with solvent oil b.p. 60°-120°C for 3 hours at 65°C in the ratio 1:6, wt/vol. The oil was removed, the pH of the mixture was raised to 8.0 with 6% ethanolic NaOH and the mixture was refluxed for 3 hours at 65°C. After reflux the product was neutralized with hydrochloric acid and the supernatant liquid was decanted off. The residue was filtered and shaken with petroleum ether in the ratio of 1:3. The product obtained after decantation was kept at 50°C for desolventization.

III. MATERIALS AND METHODS

Materials

Two members of the family Elasmobranchii were used in the study. Most of the work was done with spiny dogfish, Squalus acanthias, while sand shark, Carcharias taurus was used as the raw material for two experiments. These two species were readily available from the fishermen at Point Judith, Rhode Island, U.S.A. The spiny dogfish was collected in two batches, one in the last week of March and the second in the last week of April, 1971. The batch of sand shark was collected in the first half of September, 1971.

One consignment of spiny dogfish and the consignment of sand shark were washed to remove loose dirt and processed by skinning, beheading, gutting and filleting on the date of their receipt. The flesh, with cartilages, from one batch of dogfish was minced by a hand-driven meat grinder and stored in a deep-freeze at -20 °F for further processing to FPC. The flesh from sand shark, without cartilages, was ground in a motor driven "Hobart Food Cutter". Half the ground flesh from the sand shark was divided into four portions for studying the preservation of ground meat with 95% ethanol. The other half of ground flesh was used to study the effect of different concentrations of sodium hexametaphosphate in FPC production.

The second consignment of spiny dogfish was cleaned with tap water and put into the deep-freeze over the weekend. The fish were partially thawed and processed like the dogfish described above, except that the

flesh, without cartilage, was ground in the motor driven food chopper. The minced flesh was preserved in the deep-freeze for future use.

Methods

A. Methods of Processing

(1) Crosscurrent Solvent Extraction The principle for batch cross-current solvent extraction of fish muscle is shown in Figure 1. Essentially it consists of extracting a material with successive portions of fresh solvent. Two types of solvents were used separately for extraction. The solvents were 95% ethanol (b.p. 78.3 °C) and commercial hexane (b.p. 65 -69 °C). Two hundred grams of ground fish was well mixed with 200 ml of 95% ethanol and heated to 65 ± 5 °C with stirring. When the temperature was attained, the slurry was held at that temperature for 15 minutes with occasional stirring. The hot mixture was subjected to filtration (F). The filtrate is termed miscella (M), and the residue is termed cake (C). And the whole process from mixing of flesh with solvent through the filtration operation is called the first stage.

The cake (C-1) was then mixed with another 200 ml 95% ethanol, heated to 65 ± 5 °C, and held at that temperature for 15 minutes with stirring. The mixture was filtered. The filtrate is termed miscella M-2, the residue is cake C-2 and the whole process is the second stage. In a similar fashion, the cake could be extracted with additional batches of solvent. After completing extraction with selected solvents, the fish cake was desolventized and ground in a Wiley Inter-

mediate Mill with 60 mesh screen. Solvents and oils are to be recovered from miscellas.

(2) Countercurrent Solvent Extraction The batch countercurrent solvent extraction principle is shown in Figure 2. In a process where three successive extractions with a solvent are needed, the first, second and third extractions are termed as first, second and third stage, respectively. Each of the vertical columns represents a stage. One batch of fish processed through three stages is termed a run. In three-stage countercurrent processing, true countercurrent extraction does not start until after the third run.

After establishment of true countercurrent process extraction, a batch of raw material is extracted with the miscella from the second extraction of the previous run. The mixture is held at $65^{o} \pm 5^{o}$ C for 15 minutes with stirring and then filtered. The filtrate is miscella M-1 and residue is wet cake C-1. The wet cake is extracted with miscella M-3 from the previous run at a temperature of $65^{o} \pm 5^{o}$ C for 15 minutes. The mixture is filtered. The filtrate is second miscella M-2, and the wet cake C-2 is extracted with fresh solvent as described for the previous two stages. The mixture is filtered. The filtrate is miscella M-3, and the wet cake C-3 is subjected to further countercurrent extraction, or desolventized by air drying and ground to FPC. The first miscellas (M-1) would be sent to solvent recovery units to recover lipids and respective solvents.

In Figure 2, CA-1, CA-2 and CA-3 are wet cakes and MA-1, MA-2 and MA-3 are miscellas in run A of stages 1, 2 and 3, respectively. CB-1, CB-2, and CB-3 are wet cakes in run B; CC-1, CC-2, and CC-3 are wet cakes in run C. MB-1, MB-2, and MB-3 are the miscellas from run B and MC-1, MC-2, and MC-3 are the miscellas from run C.

(3) Aqueous Phosphate Process The process is summarized in Figure 3. Ground fish was mixed with 1% hexametaphosphate (HMP) solution (1 g HMP per 100 ml; 100 ml per 100 g fish). The pH of the mixture, initially 6.8, was lowered to 3.6 $\pm$ 0.2 by adding 6 N H_2SO_4 with constant stirring. The acidulated mixture was heated to 65 $\pm$ 5 ^{o}C and held at that temperature for 15 minutes. The pH of the mixture was checked after the heating period by cooling a portion of the mixture to room temperature. In most cases the pH was found to be 4.0 $\pm$ 0.1 or it was adjusted to that pH by adding the required quantity of 6 N H_2SO_4. This was done to precipitate out the maximum amount of phosphate-protein complex. The mixture was centrifuged for 10 minutes at 20,000 x g in a bucket centrifuge and the supernatant liquid was decanted off. The solid was washed by mixing with water once and centrifuged as before. The supernatant liquid was decanted off and the sediment was squeezed to expel more liquid from the cake. The cake was subjected to crosscurrent or countercurrent solvent extraction using ethanol and hexane separately. After the final extraction and desolventization, the products were ground in a Wiley Intermediate Mill with 60 mesh screen.

In Figures 1-3, solvent and oil recovery steps are indicated. These were not studied in this investigation, although they would be critical steps in any economically sound FPC process.

B. Analytical Methods

The composition of raw frozen fish muscle and samples of FPC in respect to urea, protein, lipid, total volatiles (T.V.) and ash content and the water and oil holding properties of FPC were determined by the following methods:

(1) Urea Urea was estimated colorimetrically using the method described by the Association of Official Analytical Chemists (AOAC, 1970, Section 7.029). The reagent used for this purpose was DMAB (p-dimethylaminobenzaldehyde, Eastman 95). DMAB reacts with urea to form a complex which absorbs light at 420 nm.

Urea was extracted from fish muscle (1g) and samples of FPC (5gms) by shaking for 30 minutes with 80 ml distilled water, 5 ml of 10.6% $K_4Fe(CN)_6.3H_2O$, 5 ml of 22% $Zn(OAc)_2.2H_2O$ and 1 g charcoal. The mixture was filtered through a Buchner funnel. The residue was washed with 5-10 ml of distilled water and the total volume was made to 100 ml by adding distilled water. For developing color, 5 ml aliquots of the extract were mixed with 5 ml of DMAB reagent. The optical density (O.D.) of this colored solution was measured in a Beckman Model DU Spectrophotometer. The urea concentration in the sample was calculated from the observed O.D. using a standard curve.

(2) Protein Protein was determined by the macro-Kjeldahl method as described by the AOAC (1970, Section 7.016).

(3) Lipid Lipid was estimated by the semimicro method of Ambrose et al. (1968). Five gms of sample, ground freshly frozen raw fish or FPC, was blended with 30 ml chloroform, 20 ml methanol and 7 ml water for 2 minutes. Another 10 ml chloroform was added and the mixture was blended for another 30 seconds. The entire contents of the blender were transferred in a 250 ml beaker containing 4 gms anhydrous sodium sulfate and 4 gms hyflo-supercel.

The contents of the beaker were thoroughly mixed and filtered. The filtrate was transferred into a 100 ml graduated cylinder and the residue was blended with 40 ml chloroform and filtered. The filtrate was added to the original filtrate in the cylinder and 10 ml water was added to the mixture. The mixture was shaken well and allowed to settle for at least one hour with occasional slow stirring at the cylinder wall to remove adhering water. The methanol-water was removed by siphoning and a 25 ml aliquot of the chloroform layer was evaporated in a tared flask under reduced pressure. The residue in the flask was dried at 100 ^{o}C in an oven for 30 minutes. The flask was cooled to the room temperature and weighed. From the gain in weight of the flask, the percentage of lipid was calculated.

(4) Total Volatiles (T.V.) The percentage T.V. was calculated by drying the sample at 105 ^{o}C for 18 hours as described by the AOAC (1970, Section 24.003).

(5) Ash The ash content was estimated by igniting the sample at 525 ^{o}C to a constant weight as mentioned in the Official Methods of Analysis (AOAC, 1970, Section 18.012).

(6) Yield The yield of FPC was calculated according to the following formula:

$$\% \text{ yield} = \frac{(\text{wt FPC produced})\ (100)}{\text{wt ground fresh frozen raw meat}}$$

(7) Water Holding Capacity Two gms of FPC was shaken with 25 ml tap water for 2 minutes and centrifuged at 480 x g for 5 minutes. The supernatant liquid was decanted off. The wet residue was weighed and the percentage of water held was calculated.

(8) Oil Holding Capacity Two gms of FPC was shaken with 25 ml of corn oil for 2 minutes. The mixture was centrifuged at 480 x g for 5 minutes. The supernatant oil was decanted off, the residue was weighed and the percentage of oil held was calculated. The water and oil holding capacities were studied following Jayatilleke (1971).

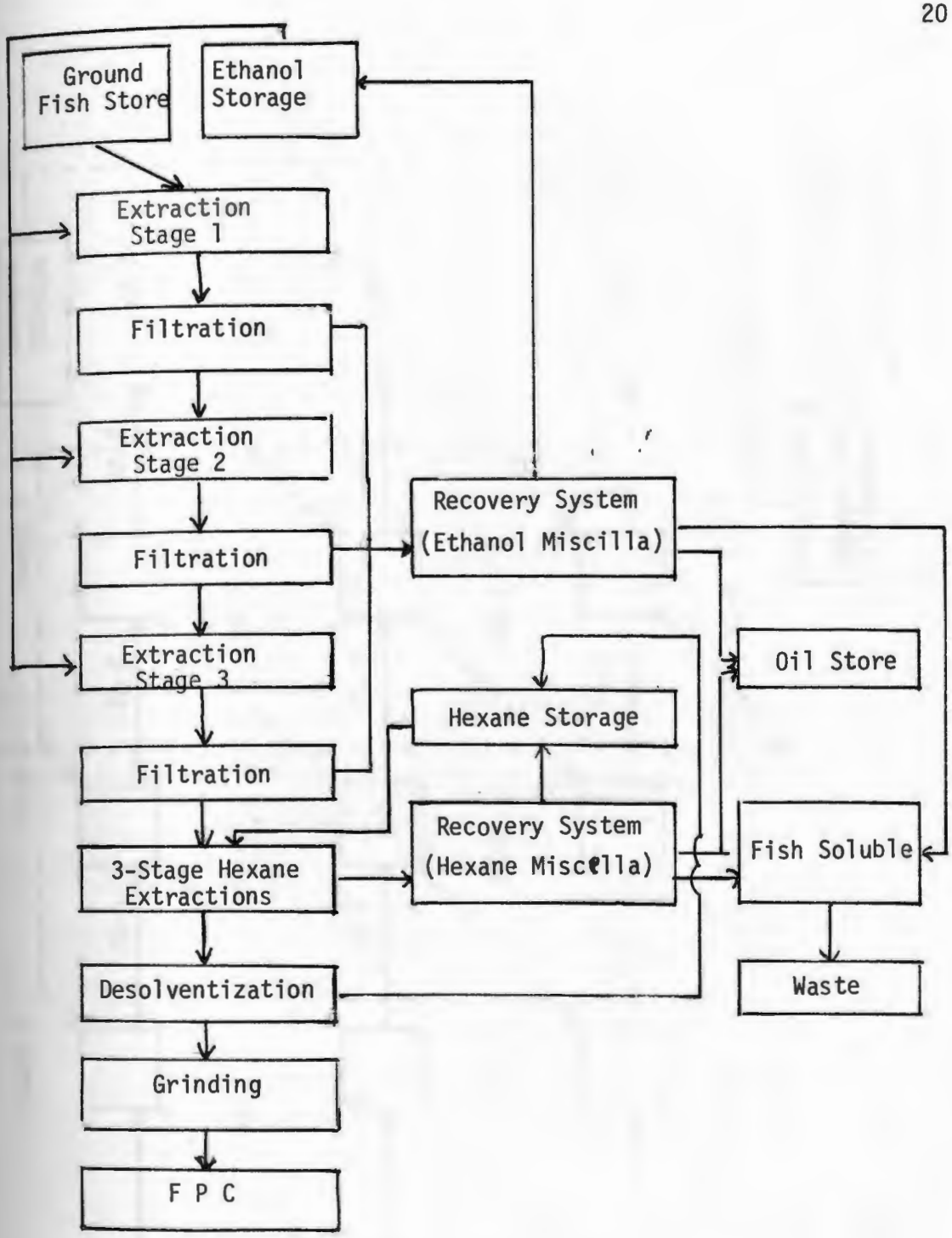

Figure 1. Schematic Diagram of 3-Stage Batch Crosscurrent Solvent Extraction Process

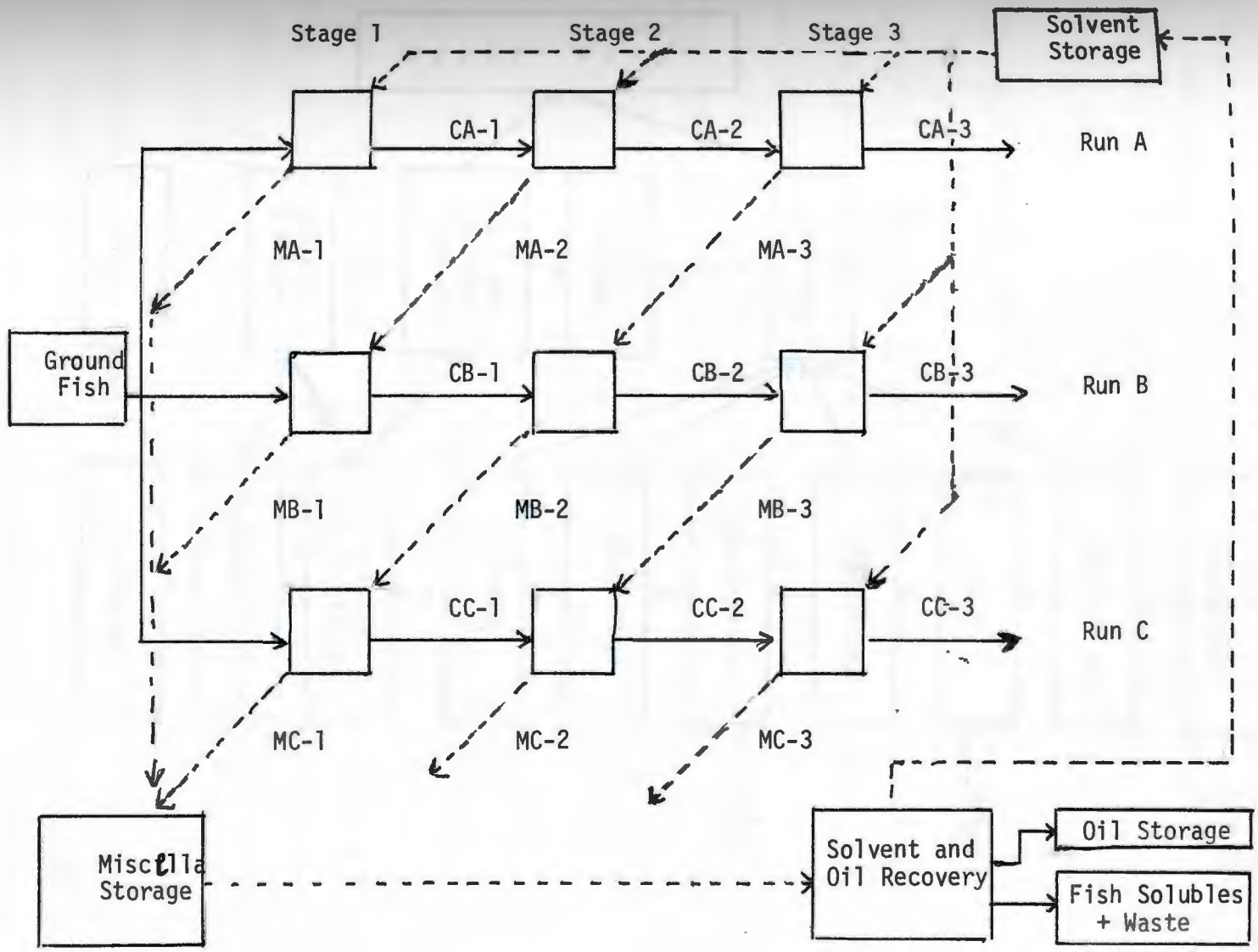

Figure 2. Schematic Diagram of 3-Stage Batch Countercurrent Extraction Process

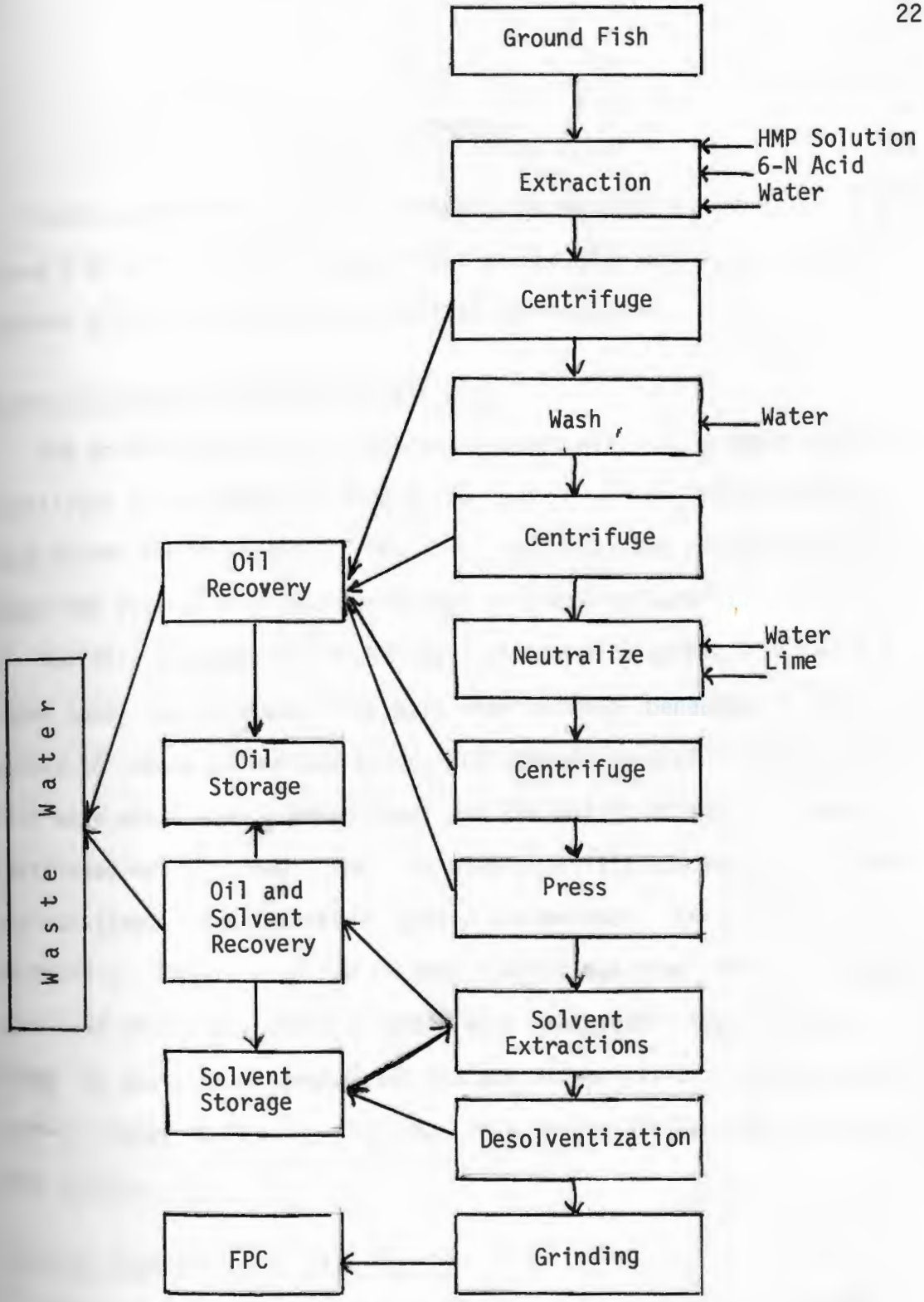

Figure 3. Schematic of Aqueous Phosphate Process

IV. EXPERIMENTAL

Unless otherwise specified, extractions were performed at 65 $^{o}_{-}$ 5 ^{o}C, using 1 ml of solvent per gram of wet ground fish and stirring for 15 minutes after attaining the extraction temperature.

Ratio of Processable Meat to Whole Fish

The percentage yield of processable meat with and without backbone cartilages in one batch of five spiny dogfish and one batch of four sand sharks was worked out. This also indicated the relationship between the size of fish and percentage of processable meat.

For this purpose, the fishes were washed with water, wiped with a paper towel and weighed. They were then skinned, beheaded, gutted, washed to remove slimes and blood, and allowed to drain. The processed fish were wiped with a paper towel and the weight of each fish with cartilages was recorded. The fish were next filleted and the fillets and cartilages were separated, pooled and weighed. The data are shown in Table 1.. While the yield in both species was about 50%, it appears that sand sharks may yield slightly more processable meat than dogfish. It was also observed that the percentage yield of processable meat is higher in the bigger fishes than in the smaller fish of the same species.

Chemical Composition of Fish Muscle

The percentage composition of two consignments of spiny dogfish and one consignment of sand shark in respect to urea, total volatiles, pro-

tein, lipids, and ash was determined and recorded in Table 2. The urea contents of two batches of spiny dogfish did not vary significantly but there was considerable difference in their lipid and moisture contents. The sand shark sample contained more protein and urea than the spiny dogfish samples.

Effect of Temperature on Urea Extraction

The effect of temperature (25°, 45°, and 60°C) on extractability of urea, lipids and moisture has been studied. The experiment was conducted on the crosscurrent principle using 95% ethanol and commercial hexane as solvents. In one run, extraction with hexane was omitted to compare lipid extraction with the runs in which both solvents were used. The FPC's thus produced were analyzed and the results are presented in Table 3. More urea was extracted with an increase in temperature but there was no difference in lipid extractability in small range of change of temperature (25° and 45°C). Considering the above observation, it may be inferred that hexane has a marked capability for extracting lipids, since the product prepared without hexane contained 14.2% lipid as compared with less than 1.5% after one hexane wash.

Effect of Ethanol Concentration on Urea Extraction

The effect of the concentration of ethanol used in the first stage on urea removal is shown in Table 4. The initial extraction with different ethanol-water ratios was followed by two 95% ethanol and two hexane stages. Extractions were crosscurrent. The 20% ethanol treat-

ment was most efficient in extracting urea (0.040%), while the yield of FPC was also lowest, an indication of greater proteolytic enzyme activity than in the 40-95% ethanol treatments.

Effect of pH on the Processing of Shark Meat by the Aqueous Phosphate Process

In this series of experiments the initial aqueous extractions were conducted at pH 5.0, 4.5, 4.0, and 3.5 and at temperatures of 25°C and 45°C to show the combined effect of temperature and pH on extractability of urea in the presence of 1% hexametaphosphate (HMP). The filter cakes obtained after one wash of the HMP-precipitate were extracted twice with 95% ethanol to observe the dehydrating and lipid extracting ability of the ethanol. From the chemical analysis of the products, Table 5, it can be seen that the urea content of each sample was low (0.060-0.082%) at 25°C and even lower (0.024-0.035%) at 45°C. The lipid contents were very high, while total volatiles were higher than usual for FPC.

Effect of HMP Concentration on Yield of FPC

Ground shark meat was treated with 0.5, 1.0, 2.0, and 4.0% of HMP. For the 0.5% treatment, 1 g of sodium hexametaphosphate was dissolved in 200 ml water. This solution was added with stirring to 200 g of fish in a beaker. The mixture was adjusted to pH 4.0 ± 0.1, heated to 65°C and stirred at that temperature for 15 minutes. The slurry was centrifuged, the supernatant was decanted and 200 ml of water was added to the sediment. After mixing the slurry was centrifuged. The solid residue was pressed to remove more liquid and the press cake

was extracted twice with 95% ethanol and twice with hexane, crosscurrently. The products were analyzed and are reported in Table 6.

It appears that the urea content in the FPC samples was pretty low. The level of total volatiles was still high (11.6-12.7%). There was not much difference in yield in the samples processed with 1, 2 and 4% HMP, but the yield was slightly lower in the case of the sample treated with 0.5% HMP. The lipid contents of all the samples of FPC were within the FDA limits.

Countercurrent Solvent Extraction of Ground Meat to Produce FPC

A comparison of extractability of urea, lipid and moisture by crosscurrent and countercurrent solvent extraction was conducted. Three ethanol and two hexane stages were employed in one set and three ethanol and three hexane stages were used in another set of experiments. The samples of FPC thus produced were analyzed and the results are tabulated in Table 7.

From the analysis of the FPC's it appears that there is no significant difference in yield or residual urea level. Total volatiles were within the acceptable limit. However, the lipid content for FPC produced by the countercurrent process using three ethanol and two hexane extractions was higher than the FDA limit. In other samples it was within the acceptable limit. It is noted that the starting material used for this comparison and in several subsequent experiments contained 13.4% lipid.

[redacted]t of Varying the Number of Stages in the Countercurrent Extraction [redacted]hod

The FPC's produced with several combinations of ethanol and hexane stages in the countercurrent mode were analyzed and the results are tabulated in Table 8. It was observed that three ethanol stages followed by three hexane stages produced the desired result. The urea content could not be lowered below 0.145% even extracting with a third ethanol stage. The urea content in FPC produced by two stages of ethanol and three stages of hexane was more than double that with three stages of ethanol and two stages of hexane. Lipid and moisture contents were brought down to acceptable values where three stages of extraction with hexane were used. No appreciable difference in yield was observed.

[redacted]mbination of Aqueous Phosphate Process and Countercurrent Extraction

The analytical results of FPC produced by aqueous phosphate process in combination with various stages of extraction with ethanol (95%) and hexane are presented in Table 9.

It was observed that extraction of the aqueous phosphate process cake in three ethanol stages followed by three hexane stages produced excellent results. The urea level was brought down to 0.067% as compared with 1.1% in raw meat. The moisture and lipid levels were reduced to 8.43 and 0.56%, respectively. The yield was also good.

[redacted]ing Quality of Ground Shark Meat in 95% Ethanol

The sand shark meat without cartilages was preserved in 95% ethanol using one ml ethanol per gram of meat. Samples were stored at room

temperature (28 ± 2 °C) for 24 hours, one week, and 4 weeks. There was also a control sample at zero hour. All were processed by crosscurrent solvent extraction using two additional ethanol and three hexane stages. The composition of the samples of FPC were determined and are presented in Table 10.

The yield was very close in each of the four samples. There were no observable differences in odor, color, texture, or taste of the samples (Table 12).

Sensory and Functional Properties of FPC

The water and oil holding capacities of FPC samples prepared by crosscurrent, countercurrent, and aqueous phosphate process, and the samples on which keeping quality in ethanol was studied were determined. Corn oil was used in studying the oil holding capacity. The results are placed in Table 12. Observations of color, odor, texture, and taste are also presented in Table 12.

Length of Extraction Period

Ground dogfish meat was extracted for 15, 30 or 60 minutes per stage in the crosscurrent fashion with various combinations of ethanol and hexane stages. In all cases the first extraction was made with 47.5% ethanol to minimize the urea content of the product. The results are presented in Table 11. It was observed that the FPC produced with six (3 ethanol and 3 hexane) 15 minute extractions was comparable in lipid content (0.74%) with FPC produced with five (3 ethanol plus 2 hexane or 2 ethanol plus 3 hexane) 30 minute ex-

tractions or five 60 minute extractions with ethanol alone. The best result on lipid removal was obtained when the fish was processed with two ethanol followed by three hexane stages, extracting for 60 minutes in each stage. The lipid content of the FPC was 0.25%, but the urea content was 0.40%. The fish processed by three ethanol followed by two hexane stages for 60 minutes per stage yielded FPC with 0.45% lipid and 0.128% urea, while FPC prepared with six 60 minute ethanol extractions had only 0.41% lipid and no detectable urea.

Table 1

Estimation of Processable Meat from Whole Fish

Species	Weight of Whole Fish g	Weight of Muscle with Cartilages g	Percentage of Whole Fish		
			Muscle with cartilages	Muscle	Cartilages
Spiny dogfish	320	100	31.2		
	920	440	47.8		
	1110	580	52.2		
	1240	640	51.6		
	1480	632*	42.9*		
Pooled sample	5100	2395	47.0	44.2	2.8
Sand shark	745	365	49.0		
	865	465	53.8		
	930	505	54.3		
	965	510	52.84		
Pooled sample	3505	1845	52.6	49.6	3.0

*Some loss was incurred during processing.

Table 2

Chemical Composition of Ground Shark Meat

Material	Urea %	T.V.* %	Protein** %	Lipid %	Ash %
Spiny dogfish with cartilages	1.10	75.0	15.6	4.9	1.61
Spiny dogfish without cartilages	1.08	70.6	16.0	13.4	0.92
Sand shark without cartilages	1.44	74.5	17.4	4.0	1.12

*T.V. = total volatiles
**Crude protein corrected for urea nitrogen

Table 3

Effect of Temperature on the Crosscurrent Solvent Extraction of Urea from Shark Meat

Number of Extractions EtOH	Hexane	Temperature °C	Composition of FPC* Urea %	Protein %	Lipid %	T.V. %	Ash %
2	1	25	0.61	88.5	1.3	14.5	5.8
2	1	45	0.52	87.3	1.4	13.8	5.7
2	0	60	0.10	68.8	14.2	12.7	4.3

*Raw material, dogfish, with cartilages, contained 1.10% urea and 4.9% lipid.

Table 4

Crosscurrent Solvent Extraction of Shark Meat at 65°C
Using Ethanol in Diminishing Order of
Concentration for First Stage

% EtOH in first *	Yield of FPC	Composition of FPC**				
		Urea %	Protein %	Lipid %	T.V. %	Ash %
95	12.4	0.104	86.0	1.7	6.8	4.1
80	13.0	0.092	88.3	1.9	6.7	4.3
60	12.7	0.084	88.3	2.4	6.4	3.6
40	13.1	0.088	90.1	2.1	6.6	5.9
20	11.6	0.040	88.6	1.9	6.5	3.6

*Filter cake extracted with 2 additional ethanol and 2 hexane stages.
**Raw material was dogfish containing 1.1% urea and 4.9% lipid.

Table 5

Effect of pH and Temperature in Aqueous Phosphate Process in Combination with Two-Stage Crosscurrent Ethanol Extraction

[redacted]	Temperature °C	Composition of FPC*				
		Urea %	Protein %	Lipid %	T.V. %	Ash %
5.0	25	0.060	68.0	12.6	14.8	4.6
	45	0.033	70.5	11.0	13.8	4.7
4.5	25	0.078	58.9	20.7	14.9	5.5
	45	0.024	64.6	15.5	14.5	5.2
4.0	25	0.082	69.6	12.5	13.9	5.0
	45	0.034	63.1	18.1	12.7	6.1
3.5	25	0.072	72.0	10.6	12.0	5.4
	45	0.035	66.3	17.2	11.0	5.5

*Raw material was dogfish with cartilages, 1.1% urea, 4.9% lipid.

Table 6

Treatment with Various Concentrations of Sodium Hexametaphosphate (HMP) at pH 4.0 by Aqueous Phosphate Process* in Production of FPC

HMP Concentration g/100 g of [illegible]ed fish	Yield of FPC %	Composition of FPC**				
		Urea %	Protein %	Lipid %	T.V. %	Ash %
0.5	16.4	0.049	85.0	0.42	12.3	1.8
1.0	16.9	0.049	85.2	0.28	12.3	2.6
2.0	17.2	0.053	81.5	0.17	11.6	3.4
4.0	17.1	0.049	83.1	0.15	12.7	4.4

*HMP precipitate received one aqueous wash followed by two ethanol and three hexane crosscurrent extractions.
**Raw material was sand shark containing 1.44% urea and 4.0% lipid.

Table 7

Comparison of FPC from Crosscurrent and Countercurrent Solvent Extraction of Shark*

Method of Extraction	Number of Stages EtOH	Hexane	Yield of FPC	Composition of FPC* Urea %	Protein %	Lipid %	T.V. %	Ash %
Crosscurrent	3	2	13.4	0.162	87.7	0.53	8.59	2.32
Countercurrent	3	2	13.1	0.160	88.4	0.90	9.68	2.29
Crosscurrent	3	3	14.0	0.140	92.5	0.47	4.90	2.20
Countercurrent	3	3	12.5	0.145	89.6	0.61	9.40	1.96

*Raw material was dogfish containing 1.08% urea and 13.4% lipid.

Table 8

Effect of Variation in Number of Stages in Countercurrent Extraction of Shark Meat for Production of FPC

Number of Stages		Yield of FPC %	Composition of FPC*				
[redacted]	Hexane		Urea %	Protein %	Lipid %	T.V. %	Ash %
2	2	14.0	0.340	87.9	1.73	10.2	2.23
2	3	12.9	0.380	89.8	0.62	8.8	2.32
3	2	13.1	0.160	88.4	0.90	9.7	2.29
3	3	12.5	0.145	89.6	0.61	9.4	1.96

*Raw material was dogfish containing 1.08% urea and 13.4% lipid.

Table 9

FPC Produced by Aqueous Phosphate Process with Variation in Number of Stages of Countercurrent Solvent Extraction

Number of Stages		Yield of FPC	Composition of FPC*				
EtOH	Hexane	%	Urea %	Protein %	Lipid %	T.V. %	Ash %
2	2	12.4	0.115	86.5	2.10	7.2	2.87
2	3	12.4	0.116	86.6	1.06	7.6	2.82
3	2	12.7	0.067	84.2	1.52	8.5	3.55
3	3	12.7	0.067	88.2	0.56	8.4	3.83

*Raw material was dogfish containing 1.08% urea and 13.4% lipid.

Table 10

Keeping Quality of Ground Shark Meat in 95% Ethanol at 28±2 °C

Period of Preservation	Yield of FPC %	Composition of FPC*				
		Urea %	Protein %	Lipid %	T.V. %	Ash %
0 hours	16.6	0.131	87.3	0.15	12.7	1.98
24 hours	17.4	0.162	87.6	0.08	14.0	1.88
1 week	16.8	0.181	84.9	0.13	11.1	1.98
4 weeks	16.0	0.162	90.4	0.20	8.1	1.98

*Raw material was sand shark containing 1.44% urea and 4.0% lipid. Crosscurrent extractions with 2 additional ethanol and 3 hexane stages.

Table 11

Effect of Varying Length of Extraction Period in Production of FPC from Shark Meat by Crosscurrent Solvent Extraction

Sample	Period of extraction Minutes	Number of Stages EtOH	Hexane	Yield of FPC %	Composition of FPC* Urea %	Protein %	Lipid %	T.V. %
1	15	3	3	13.7	0.124	93.1	0.74	5.06
2	15	5	0	13.2	0.025	89.3	4.04	5.85
3	15	6	0	13.2	0.017	92.9	3.20	6.02
4	30	2	2	14.8	0.504	94.6	1.65	4.65
5	30	2	3	15.2	0.400	92.9	0.78	5.02
6	30	3	2	16.2	0.156	95.2	0.87	4.72
7	30	3	3	15.1	0.184	93.4	0.57	4.82
8	30	5	0	14.7	0.033	88.1	3.19	6.35
9	30	6	0	14.7	0.017	90.8	1.50	6.49
10	60	2	2	13.8	0.396	95.1	1.21	4.66
11	60	2	3	13.6	0.400	92.4	0.25	4.93
12	60	3	2	14.0	0.128	93.8	0.45	4.64
13	60	5	0	12.0	0	93.8	0.67	6.08
14	60	6	0	12.7	0	93.8	0.41	6.10

*Raw material was dogfish containing 1.08% urea and 13.4% lipid.

Table 12

The Sensory Evaluation and Functional Properties of FPC from Shark

Sample no.	Method of Extraction with Stages								Remarks	
	Method	EtOH	Hexane	Color	Odor	Taste	Texture	% H_2O Held	% Oil Held	Oil %	Urea %
1	Cross-current	3	2	Light cream	Faintly amine	Slightly fishy	good	340.00	248.00	0.53	0.162
2	Counter-current	3	2	Yellow-ish cream	Odor-less	Slightly fishy	good	340.00	250.00	0.90	0.160
3	Counter-current	3	3	Whitish cream	Odor-less	Tasteless	good	340.00	298.00	0.61	0.145
4	Counter-current	2	2	Yellow	Rancid, amine	Rancid fishy	good	274.00	222.00	1.73	0.340
5	Counter-current	2	3	Light cream	Faintly amine	Fishy	good	358.00	233.00	0.62	0.380
6	A P with counter-current	2	2	Almost white	Very faintly amine	Acidic slightly fishy	good	288.00	250.00	2.10	0.115
7	A P with counter-current	2	3	Almost white	Very faintly amine	Acidic slightly fishy	good	287.00	270.00	1.06	0.115
8	A P with counter-current	3	2	Almost white	Odor-less	Acidic slightly fishy	good	253.00	299.00	1.52	0.067
9	A P with counter-current	3	3	Almost white	Odor-less	Acidic slightly fishy	good	287.00	305.00	0.56	0.067

Table 12 (continued)

Sample no.	Method of Extraction with Stages			Color	Odor	Taste	Texture	% H_2O Held	% Oil Held	Remarks Oil %	Remarks Urea %
	Method	EtOH	Hexane								
10	Cross-current at 0 hours	3	3	Light cream	Very faintly amine	Slightly fishy	good	320.00	238.00	0.15	0.131
11	Cross-current after 24 hours in EtOH (95%)	3	3	Light cream	Very faintly amine	Slightly fishy	good	295.00	265.00	0.08	0.161
12	Cross-current after 1 week in EtOH (95%)	3	3	Light cream	Very faintly amine	Slightly fishy	good	303.00	256.00	0.13	0.181
13	Cross-current after 4 weeks in EtOH (95%)	3	3	Light cream	Very faintly amine	Slightly fishy	good	319.00	216.00	0.20	0.161

Table 13

Proximate Composition of FPC Produced by Different Organizations

[redacted]**	% Protein	% Lipid	% T.V.	% Ash
I	81.05	0.24	3.60	14.98
II	90.70	0.30	3.25	8.13
III	90.80	0.39	9.95	2.09
IV	89.60	0.61	9.40	1.96

*Source: E. M. Nikkila, M. S. Thesis, University of Rhode Island, 1972.

**I. Instant Protein (trade name) prepared from red hake by Alpine Marine Protein Industries, Inc., New Bedford, Massachusetts, following the Viobin Process.

II. Prepared from eviscerated herring using IPA as solvent by Astra-Nutrition AB, Sweden.

III. Prepared from deboned hake using three stage counter-current extraction with methanol in laboratory of Dr. T. L. Meade, University of Rhode Island.

IV. Prepared from dogfish in Food and Resource Chemistry Laboratory of the University of Rhode Island using countercurrent extraction with three stage ethanol followed by three stage hexane (project under review).

V. DISCUSSION

Dr. Samuel Johnson defined fish (Pariser, 1971) as any animal that inhabits the water, but strictly speaking, fish is the term given only to a large class of vertebrates including the bony and cartilagenous fishes. The Elasmobranchii belong to the latter group and sharks belong to the Elasmobranchii. So, the protein concentrate prepared from shark meat can be named "Fish Protein Concentrate". Skates and rays, which also contain urea (Simidu, 1961), and would require identical processing would be excluded by the term "Shark Protein Concentrate". Therefore, it is suggested that the product described in this thesis be named "Fish Protein Concentrate".

Solvents—General Considerations

The selection of solvents is greatly dependent on their ability to extract urea, moisture and lipid. Urea is highly soluble in water, soluble in alcohols, but insoluble in hydrocarbons (Weast, 1969). Water is the most suitable solvent to extract urea, but it also dissolves and removes some protein, leading to a loss of nutrients and reduction in yield, which increases the cost of production and price of the product and decreases the profit margin. Water does not extract lipids. Alcohols extract water from biological tissue. The solvents which are to be selected for FPC production must be available in abundance, or at least there must be potential sources of the materials from which they can be manufactured. In Western countries,

isopropyl alcohol (IPA) has found its way into the FPC industries, isobutanol has been suggested as a solvent in Chile (Hevia, 1971) and ethanol has been proposed for the producing of FPC in India (Moorjani and Lahiry, 1970). There is a distillery in the Peoples' Republic of Bangladesh (generally known as Bangladesh) where ethanol is manufactured. This industry could be expanded to fulfill the solvent requirements of an FPC industry in Bangladesh.

Alcohols are not as efficient as the low boiling hydrocarbons for extracting triglycerides. Among the hydrocarbons hexane is relatively cheap and readily available. There is also a mineral oil refinery based on imported crude oil in Bangladesh. So hexane might be available in our country. Hexane may be removed easily during desolventization under reduced pressure and temperature without causing any appreciable damage to the nutritive value of FPC. It is used in Peru for the production of FPC from fish meal. Neither it nor ethanol is toxic when ingested in very small doses as might be retained as residual solvent in FPC. Hence, ethanol and hexane have been selected for the production of FPC from sharks.

Urea—Source, Effect and Fate

The urea content of one batch of spiny dogfish was 1.10% and the second batch contained 1.08%, whereas the urea content of sand shark was 1.44%. These results agreed with the findings of previous workers. Simidu (1961) reported that sharks caught in the Japan sea contain 1-2.3% urea in their muscle. Alverson and Stansby (1963) reported that spiny dogfish contains urea in its muscle, but they did

not mention it quantitatively. Osterhaug (1961) reported that the urea content of dogfish may exceed 2.5%. All of them pointed out that urea plays an important role in osmoregulation in Elasmobranchii. West and Todd (1963) pointed out that when certain proteins are dissolved in strong urea solution, their molecular weight becomes less, indicating the rupture of loose linkages (non-peptide) and formation of smaller particles. This finding may be utilized in solving the problem of osmoregulation in Elasmobranchii. The same author also pointed out that urea is the end product in protein metabolism in humans and a typical 68 kg subject had 5.74 g of urea nitrogen in his body pool, which represented a urea space of 33.8 liters. In the human, urea usually represents 80-90 percent of the total urinary nitrogen. So, the small residual urea in FPC will not be a physiological problem to humans or other animals. Neither human nor any other mono-gastric animal can utilize urea nitrogen for the purpose of synthesizing protein as the cattle do (Osterhaug, 1961).

Suyama and Tokuhiro (1954b) reported that decomposition of muscle urea in C. melanopterus began as low as 80 °C, the amount of urea and the intensity of the biuret reaction decreasing with increasing temperature. Osterhaug (1961) reviewed reports in the Fishing Gazette of 1917, concerning canned shark meat. In the report, it was pointed out that after shark meat had been in the can two or three months, the flesh became soft and flabby and fairly reeked with the pungent oil, making them positively nauseating. She also cited in the Pacific Fisherman of 1917 that progressive detinning of the interior of cans

by ammonium hydroxide obtained from urea results in the absorption of tin by the fish and renders it unsuitable for food. These findings indicate the unsuitability of Elasmobranchs in the canning industry.

Simidu et al., (1952) measured NH_3 production during storage of shark flesh. It occurs in two stages, the first due to urease present in the muscle and the second due to urease produced by putrifying bacteria. The activity of urease preparation extracted at intervals during storage was proportional to the NH_3 content of the muscle. In the second case the NH_3 production was proportional to the growth of microorganisms. Both the enzymatic and microbial spoilage of fish of the family Elasmobranchii occurs in raw meat in storage, if the temperature is not low enough to stop enzymatic action.

The above mentioned problems may be overcome by producing FPC. During processing, the enzymatic activity can be destroyed, the growth of microbes can be retarded, and the urease substrate, urea, can be lowered substantially.

Urea Removal

The removal of urea from shark meat was tested with simple solvent extraction and aqueous phosphate methods. The effect of varying temperature, concentration of ethanol in the first stage, and number of stages on extraction of urea from shark meat was studied. The comparison between crosscurrent and countercurrent extraction of shark meat in respect to extractability of urea was performed.

It was observed (Tables 3 and 5) that solubility and removal of urea from shark meat by ethanol and water increased with an increase in temperature.

The urea contents of FPC samples were 0.61, 0.52 and 0.10% at 25, 45 and 60°C, respectively, after two crosscurrent ethanol extractions of ground dogfish (Table 3). At the time of performing this set of experiments a very useful observation was made. It was found that the slurries produced at 45° and 25°C were viscous and it was very difficult to separate miscella from the cake by filtration or pressing. But at 60°C, the protein in the slurry was stiffer on account of coagulation of muscle protein and it was easier to separate miscella from cake by either filtration or pressing.

Since Suyama et al., (1954) noted that urea starts decomposing at 80°C and muscle protein is denatured at 60°C, it was decided to conduct other experiments within the range of 60-70°C.

With the aqueous phosphate process plus two crosscurrent ethanol extractions (Table 5), urea residues were reduced to 0.082 and 0.034% at temperatures of 25 and 45°C, respectively.

An experiment was set up to observe the effect of different concentrations of ethanol (20%, 40%, 60%, 80% and 95%) on extraction of urea from shark meat for the production of FPC. The results are listed in Table 4. It was observed that 20% ethanol was most efficient in extracting urea but the FPC production was lower than with other concentrations. There was no marked difference in urea extracting capability between 40-90% ethanol.

The concentrations of urea in FPC produced by crosscurrent and countercurrent solvent extraction methods were not significantly different. This can be seen in Table 7.

The urea concentration in FPC produced from dogfish meat by changing the number of extraction stages is noted in Table 8. It was observed that urea concentration with two ethanol stages plus two or three hexane stages were 0.34 and 0.38%, whereas the values were 0.16 and 0.14% in FPC prepared with three ethanol stages and two or three hexane stages. When FPC was prepared using five stages of extraction with ethanol there was only a trace of residual urea (Table 11). With six ethanol stages, no urea was detected in the FPC.

In a later experiment at 65°C (Table 9), the aqueous phosphate process in combination with three countercurrent ethanol followed by three countercurrent hexane extractions yielded FPC with 0.067% urea, lower than either crosscurrent or countercurrent solvent extraction alone.

Lipids

The Food and Drug Administration (FDA) of the U.S.A. has prescribed the limit of lipid content in FPC at 0.5%. It has been observed that if the lipid content is allowed to exceed that limit, the FPC may get rancid. So lipid causes a problem in FPC.

The lipids in aquatic animals as a whole are characterized by a high degree of unsaturation. In the elasmobranchs, alkozyglyceride and squalene partly or entirely take over the function of triglyceride (Lovern, 1962). Olcott (1962) pointed out that highly unsaturated lipids of fish are readily susceptible to attack by molecular oxygen. The reaction proceeds by a free radical mechanism and is, therefore, characterized by an induction period followed by an accelerating rate

of oxygen absorption with concurrent development of peroxides, rancid odor and polymerized products. The rate of initiation of free radical reactions is increased by heat, light, irradiation and heavy metals. Free radicals react with oxygen to yield peroxy radicals, which then abstract hydrogen from the substrate yielding hydroperoxides and new free radicals. A single chain is thus continued, but new chains result from the breakdown of hydroperoxides to give new free radicals. Ultimately a plethora of reaction products results. Lea (1962) reports that lipoperoxides, aldehydes, acids, ketohydroxy and epoxy compounds cause off-odors and flavors. These are the autoxidation products from unsaturated fats. Off-odors and flavors make the products unacceptable to the consumers. So the FDA limited the lipid content in FPC as mentioned earlier.

Joslyn (1970) emphasized that the following factors are involved in completeness of extraction of lipids:

1. Nature of the material to be extracted
    a. Comparative rates at which the components pass into solution
    b. Effect of one component upon the solubility of the other
    c. Size of the particles of which the mixture is composed
    d. The relative amounts of the more and less soluble compounds
2. Nature of solvent
    a. Diffusibility
    b. Solvent power

3. Surface offered to the solvent
4. Rate at which the solvent circulates through the extraction shell
5. Relative amounts of solvent and material to be extracted

He also emphasized that the development of off-flavor has been ascribed to the degradation of lipids either catalyzed by enzymes or initiated by autoxidation.

Lipid in Raw Materials

Three batches of fish were used as raw material in these experiments. There were two batches of spiny dogfish with lipid contents in the meat of 4.9 and 13.4% and one batch of sand shark with 4.0% lipid in meat (Table 2). On a dry basis these amount to 19.6, 45.5 and 15.7%, respectively.

Lipid in FPC

The lipid content of FPC depended upon the lipid content of the starting material. When the lipid content of the raw fish was 13.4%, the FPC contained 0.54% lipid, while the lipid content of FPC was below 0.2% when the lipid in the raw material was 4.0% (Tables 7 and 10).

It appears from Table 7 that the countercurrent extraction was not as efficient in removing lipid as the crosscurrent extraction. Also lipid levels were not lower in FPC produced by the aqueous phosphate process in combination with countercurrent solvent extraction than the FPC produced by countercurrent solvent extraction alone (Tables 8 and 9).

The extraction of lipid was better with longer periods of extraction. This can be observed from the values of lipid from the samples 1, 7, 12; 2, 8, 13 and 3.9, 14 of Table 11. It appears from samples 11 and 12 that the number of stages of extraction can be reduced from six to five in crosscurrent method using combined solvents by increasing the time of extraction to 60 minutes. Lipid was reduced to the acceptable limit by 6-stage crosscurrent solvent extraction using only ethanol with the extraction period of sixty minutes in each stage. This experiment suggests that a multistage countercurrent extraction using only ethanol as solvent should be conducted.

It appears that hexane has a marked effect on extraction of lipids of shark muscle after dehydrating the meat; the lipid content in FPC was reduced to 1.4% from 14.2% with one extraction at 65 ^{o}C (Table 3).

It was observed that the FPC having higher lipid content (1.73%) was more yellow in color, more rancid in odor and more fishy rancid in flavor than any of the FPC samples containing less lipid (Table 12).

Moisture Removal

The U.S. FDA has prescribed that the total moisture content of FPC should be less than 10%. The reason for reducing moisture content to this level is to check microbial growth and minimize the spoilage of FPC by microorganisms. Desrosier (1970) mentioned that molds can grow on food substrates with as little as 12% moisture, and some are known to grow in foods with less than 5% moisture. Bacteria and yeasts require higher moisture levels, usually over 30%. Both Carpenter (1968) and Desrosier (1970) stated that mold can grow on dry foods or other material like cloth, shoes, etc., when exposed to high humidity conditions. The latter also mentioned that enzyme activity is nil at moisture levels below one percent. So the reduction of moisture content is not to stop enzymatic spoilage but to stop or at least minimize microbial spoilage. Desrosier (1970) also pointed out that in case of fish the requirement of space in storage is reduced to about 40-50% for dry fish. But in case of FPC, the need of space in storage may be cut to about 20-25% of fresh fish or even less. The bound water content in cod fish tissues is about 9.5% (Love, 1968). It was shown by Joslyn (1970) that the rate of reduction of moisture content below 10% level is independent of the rate at which surface moisture is removed.

Moisture can be reduced from ground fish in two ways: (a) by drying and (b) by solvent extraction. Drying may harden the muscle, make it difficult to extract lipid efficiently and promote lipid oxi-

dation, rendering the product rancid. Also urea may be concentrated and undergo decomposition to ammonia giving the product off-flavor and odor. Urea and phospholipids will not be extracted in case only non-polar solvent is used to take care of the lipids. So a combination of polar and non-polar solvents was selected for this study.

The moisture contents of the raw materials used in making FPC were 70-75% for dogfish meat and 75% for sand shark meat. The moisture contents have been described as percent total volatiles (% T.V.) in our report. Total volatiles include moisture, residual solvents and other compounds which evaporate during drying. The FPC samples were air dried overnight and no solvent odor could be detected. Other volatile compounds in FPC were considered negligible in comparison to water content. Therefore, T.V. is believed to be mostly moisture.

The T.V. content in FPC produced using two ethanol stages was about 12-14% (Tables 3, 5, 6 and 10), whereas it was 5-9% in other samples (Tables 4, 7, 8, 9 and 11). These variations were possibly due to the efficiency of squeezing the cakes in different stages. Moreover, T.V. contents can be lowered by drying at reduced temperature and pressure. Under these conditions the nutritive value of FPC may not be impaired.

Toxicity of Residual Solvents

The maximum level of residual solvent allowed by FDA in FPC in case of IPA is 250 ppm and in case of ethylenedichloride, it is 25 ppm. Residual solvent in FPC produced using ethanol and hexane or ethanol alone will not be a toxicity problem. Hexane is only fatal if 50 g

is ingested at a time (Dreisbach, 1969). The effect of small doses is not known. Ethanol is commonly ingested by many people and any residue in FPC will not be harmful.

Nutritive Value of FPC

The daily dietary allowances for protein recommended by the Food and Nutrition Board, National Academy of Sciences National Research Council in 1968 for a 70 kg man (age group 22-75 years) and pregnant women is 65 g. Nikkila (1972) postulated that incorporation of 5% FPC in food (500 g) per day by some Asian and Arab countries would be sufficient to combat malnutrition. For FPC containing 0.2% urea, 25 g (5% of 500 g) of FPC would contain 50 mg of residual urea. Ingestion of this quantity of urea will not cause any harm to a man since it will be excreted from the system.

The comparative composition of some FPC's is given in Table 13. It appears that the nutrients of FPC prepared from shark are not inferior to any of the other FPC's. Geiger and Borgstrom (1962) cited the work of Ambe and Sohonie in India, who reported that shark and skate proteins in comparison to casein contain more basic amino nitrogen. They also cited the finding of Masheklar and Sohonie in respect to "essential amino acids". The latter workers found that casein was superior to shark and skate protein in threonine and tryptophan while these same fish proteins were superior to casein in arginine, isoleucine and methionine. They concluded that both shark and skate proteins are quite comparable, if not superior, to casein, as far as amino

acids are concerned and consequently could readily serve as cheap substitutes in correcting the deficiency of a number of essential amino acids, especially when the other dietary proteins are poor in lysine, arginine and cystine. So, there is no question regarding the value of shark protein in curing malnutrition. However, no work has been done in this aspect with the FPC produced from shark meat in this study.

There was no significant difference in color of the FPC produced from either dogfish or sand shark by crosscurrent and countercurrent methods, as shown in Table 12. All were cream colored except the sample produced by countercurrent process using two ethanol and two hexane extractions, which was yellow, probably due to high oil and urea content. Oil can provide aldehyde by undergoing oxidation, or Maillard reaction, to give a yellow-brown color. Most of the FPC samples had either a very faint smell of amine or were odorless. It was very difficult to distinguish the odorless samples from those with a faint amine odor. The amine odor may be due to retention of amine which was present in the meat. The sample produced with two ethanol and two hexane extractions was rancid because of the high lipid content, 1.73%.

Taste of FPC prepared by countercurrent and crosscurrent solvent extraction methods were mostly slightly fishy. This also might be due to amine. The FPC prepared by aqueous process was acidic in taste as the acid was not completely washed out. So it is suggested that the acid should be neutralized. For neutralization of acid, lime may be used. The texture of FPC was not gritty and it has been recorded

as good in Table 12. Water holding capacity of FPC prepared from dogfish and shark was as good as the FPC prepared by Jaytilleke et al., (1971). The water holding capacity of our FPC was about 250-350% and their FPC was 333%. The oil holding capacity of our sample was 200-300% whereas the oil holding capacity of their sample was 183%. At the time of conducting these experiments it was observed that both water- and oil-mixed FPC samples were soft and it should be possible to mix with any flour without any problem.

Prospect of FPC from Sharks

Market is the mother of industry. And market is established on need. The growing population needs nutritive foods. Therefore, a market is or will be available for good products. Good food products are judged by flavor, color, odor, taste and texture. As regards utilization of shark meat, Alverson and Stansby (1963) stated that the most profitable use for the carcasses would undoubtedly be as food for humans but development of this usage would be difficult. The principal objections to such development are: (1) in most parts of the U.S.A. there is a strong prejudice against eating shark, (2) sharks contain urea which would have to be removed or fixed in tissues by special processing methods before the flesh would be acceptable to most people and before canning could be accomplished, and (3) compared with many other low-priced and underutilized species of fish, the palatibility of dogfish is low. In addition, religious affairs may also be dragged in along with political implications on accept-

ability of FPC made out of shark meat. This is, of course, a part of prejudice. About 99% of the urea is removed in preparing FPC. Organoleptically, shark FPC is believed to be equivalent to FPC produced from other species of fish. The only problem then is prejudice. Shark fins are used in preparing delicious soup in Singapore, Hong Kong and some other countries. Shark meat is consumed as fish and chips in Mexico, the United Kingdom and Australia (Borgstrom, 1962). Shark meat is marketed in fresh condition to some extent in Bangladesh and also in Southern California (Ward et al., 1955). Costa Rica produces dried and salted shark meat for its own consumption (Bergstron and Paris, 1965). The people of affluent countries like the U.S.A., France or Germany may not accept it easily but the people of a country like ours should accept the product when it is not harmful to health and rather contains nutrients which they are lacking. Over and again, they have accepted shark liver oil as medicine. Thus, the only factor which will guide its way into the market is the price and a little education.

The prime objective of this project was removal of urea from shark meat in the production of FPC. The production of FPC was given second importance. The efficiency of extraction of urea, lipid and water has been described earlier. In the following section, the advantage of one complete process over the other (crosscurrent solvent extraction, countercurrent solvent extraction or aqueous phosphate process in combination with solvent extraction) will be discussed.

In a country like Bangladesh, the multistage (five or six stage) countercurrent solvent extraction process using 95% ethanol with one hour extraction period is preferred, because ethanol is manufactured in that country. Hexane would have to be imported. Of course, importing of hexane may not be a problem. The problem is maintenance of two solvent recovery systems, one for ethanol-water containing oils and trimethylamine or its oxide, and a second for hexane-ethanol-water containing oil mainly. Longer extraction periods, continuous agitation and more effective pressing of the slurry may improve the extraction process. It is suggested that more work should be done on these three issues in a pilot plant scale study to find out whether period and stage of extraction can be shortened. Shortening of either the period of extraction or the number of stages or both will minimize the cost of operation. Moreover, if the plant is based on a limited supply of raw material, the process with the long extraction period and fewer number of stages of extraction in the batches may be more suitable. But, if there is sufficient raw material and solvent to run continuously, the six-stage countercurrent ethanol-hexane procedure with fifteen-minute extraction periods may be more advantageous. Still, it is better to find out whether prolonging the extraction period in a stage or two with continuous stirring arrangement improves the efficiency of extraction of urea and lipid and also improves the quality of FPC.

The azeotropic isopropanol (AIPA) solvent extraction method for production of FPC developed by BCF is claimed to be a three stage operation. But it appears to be a four stage operation consuming about

four hours in extracting lipid and water (BCF, 1966). Hevia et al., (1971) described a simple process with isobutanol as solvent in a procedure consuming about five and one-half hours for extracting lipid and moisture. They used 3:1 solvent-fish ratio by weight in the first extraction mixing at room temperature for 30 minutes followed by reflux for four hours. After filtration the cake was washed three times using a 1:1 ratio of solvent to extracted fish. The fat content in the final FPC was 0.3%. The desolventization of wet cake, however, was not very efficient, taking about 18 hours at 60-65 ^{o}C under a reduced pressure of 25 mm of Hg. The fat content of their raw material was about 10-20% on a dry basis. It is questionable whether their method will be suitable for raw material such as shark meat which may contain 30-40% lipids.

In a country like the U.S.A. the aqueous phosphate process in combination with countercurrent ethanol followed by hexane extraction process may be suitable. As suggested by Spinelli et al. (1971), the raw materials might be processed at sea by the aqueous process and the cake and oil brought back to the shore. The cake would be processed by countercurrent solvent extraction to produce FPC. Oil may be sold before or after refining. If the whole process is conducted on the bank of a small river or a closed water mass, there is chance of water pollution on account of dumping sulfate along with organic material. Organic materials will deplete oxygen from the water mass. The sulfate may be reduced by microorganisms to hydrogen sulfide, making the water mass poisonous to other organisms.

Utilization of cartilage will increase the yield of FPC without changing the nutritive quality much. It was observed during the grinding of muscle that the cartilage blocked the screen of the grinder. The suitability of the use of cartilage in the production of FPC should be studied.

VI. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Ninety-eight percent of the urea in shark meat could be removed by use of three countercurrent or crosscurrent ethanol extractions. The urea content of the FPC was 0.16%.

2. Using the aqueous phosphate process with three countercurrent ethanol extractions, residual urea in the FPC was 0.067%.

3. With short (15 minute) extraction periods, 95% ethanol alone was not found to be a suitable solvent for extracting lipids of shark. However, with longer (60 minute) extraction periods, the fat content of dogfish muscle could be reduced to the 0.5% level prescribed by the FDA.

4. Using 3 ethanol stages followed by 3 hexane stages in the countercurrent mode, the lipid content of shark FPC was close to the FDA limit.

5. A three-stage ethanol extraction at 60-70 ^{o}C for a period of fifteen minutes in each stage reduced the moisture in fish muscle to 10% or less.

6. The efficiency of squeezing or pressing was found to play an important role in reducing the urea, lipid and moisture contents of the final product.

7. Separation of miscella from solids was much easier at 60 °C and above than at lower temperatures.

8. Filtration or pressing was much easier in later stages than in the first stage.

9. Centrifugation was observed to be essential for the aqueous phosphate process.

10. More than one water wash, and perhaps neutralization of free acid with lime may be required in the aqueous phosphate process to eliminate any acid taste.

11. Based on the bench-scale study, two methods of production of FPC from shark are recommended for Bengladesh. These are: (1) A countercurrent solvent extraction using three ethanol stages followed by three hexane stages, extracting for 15 minutes in each stage, or (2) six-stage (multistage) countercurrent 95% ethanol extraction with the extraction period of one hour in each stage. For the United States of America, the aqueous phosphate process followed by countercurrent three-stage alcohol extraction plus three-stage hexane extraction is recommended because the product was superior in respect to color, odor and texture to the products obtained by solvent extraction methods.

Recommendations for Future Work

1. Systems for recovering ethanol and hexane from respective miscellas should be studied.

2. The maximum level of residual urea in FPC at which no detectable off-smell or off-flavor develops during storage should be determined.

3. The maximum acceptable limit of TMAO and TMA in FPC should be determined so that in a reasonable storage time no bad odors and flavors are developed.

4. The PER value of the product should be determined.

5. The amino acid composition of the FPC should be estimated to get a quantitative idea about the essential amino acids. This is a requirement in food formulation.

6. By sensory evaluation tests, the acceptability of food prepared by incorporating FPC from Elasmobranchii meat should be evaluated.

7. The people in developing and underdeveloped countries should be educated by extension work through governmental machinery to accept FPC from Elasmobranchii.

APPENDIX

Prescribed Conditions of Food and Drug Administration for Use of FPC as a Food Supplement

(Federal Register 121.1202, February 2, 1967, Whole fish protein concentrate)

(1) The additive is derived from wholesome hake and hake like species of fish handled expeditiously and under sanitary conditions.

(2) The additive is used or intended for use only in the household as a protein supplement in food.

(3) The additive is packaged in consumer-sized units not exceeding 1 pound net weight.

(4) The food additive meets the following specifications:

(a) Protein content shall not be less than 75 percent by weight of the final product.

(b) Moisture content shall not exceed 10 percent by weight of the final product.

(c) Fat content shall not exceed 0.5 percent by weight of the final product.

(d) The additive shall contain not in excess of 100 parts per million fluorides.

(e) The additive shall be free of Escherichia Coli and Pathogenic Organisms, including Salmonella, and shall have a total bacterial plate count of not more than 10,000 per gram of FPC.

(f) The additive shall have no more than a faint characteristic fish odor and taste.

(g) The additive may contain residues of isopropyl alcohol and ethylene dichloride not in excess of 250 parts per million and 5 parts per million, respectively, when used as solvents in the extraction process.

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