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May 18, 2005

Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, Maryland 20740-3835

To Whom It May Concern:

This letter serves to provide the Center for Food Safety and Applied Nutrition (CFSAN) with notice that our client, Proteus Industries, Inc. ("Proteus"), has concluded that the use of a substance in further processed (or finished) poultry products is exempt from the pre-market approval requirements applicable to food additives under the Federal Food, Drug, and Cosmetic Act ("FD&C Act"), 21 U.S.C. § 301 et seq., because such use is generally recognized as safe ("GRAS"). 21 C.F.R. § 170.30.

Reference is made to our prior submission of March 3, 2004, also on behalf of Proteus. This prior notification discussed the manufacture and usage of certain fish products derived from virtually identical technology. For the sake of efficiency CFSAN may wish to consult the prior document, which we are including for your information and convenience. Also, we recognize that inspection responsibility for the manufacture of poultry products rests with USDA's Food Safety and Inspection Service (FSIS). We are therefore also providing a copy of this notification document to the FSIS Office of Labeling and Consumer Protection. Consistent with the policies of both agencies, we would encourage your efforts to coordinate your review of this request. We will be pleased to assist in this process in any appropriate fashion.

As explained below, this substance, "(species) protein", or "concentrated (species) protein" depending upon concentration, is acceptable for use in all further processed poultry products, unless specifically precluded by a recipe-type standard of identity. Moreover, to assure compliance with applicable labeling requirements, the presence of any such substance should be identified, in the appropriate order of predominance, in the ingredient statement of any finished poultry product. 21 C.F.R. § 101.4(a)(1). 9 C.F.R. 317.2(c)(2)

To ensure that your agency will be able, consistent with its established policy, to properly evaluate and respond to this notice within 90 days, the notice tracks the prescribed format and provides a description of the information that the agency considers appropriate to support a GRAS determination as set forth in the Proposed rule, "Substances Generally Recognized as Safe," 62 Fed. Reg. 18937 (April 17, 1997) (proposed 21 C.F.R. § 170.36).

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U.S. practice conducted through McDermott Will & Emery LLP.

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**1. Name and Address of the Notifier (Proposed Sec. 170.36(c)(1)(i))**

As indicated above, the product is produced by Proteus Industries, Inc. which is located at 21 Great Republic Drive, Gloucester, MA 01930. The agency should contact Dr. Stephen Kelleher at this address for further technical information regarding this notification (Telephone: (978) 675-9140 / Facsimile: (978) 675-9194 / E-mail: [skelleher@proteusindustries.com](mailto:skelleher@proteusindustries.com)).

**2. Common or Usual Name of the Substance (Proposed Sec. 170.36(c)(1)(ii))**

An appropriate descriptive name for the substance developed by Proteus for use in finished poultry products is "(species) protein", when the protein concentration is 22% protein or less, or "concentrated (species) protein" if found in concentrated form, a protein concentration greater than 22% protein.<sup>1</sup> Examples would be "chicken protein" and "concentrated turkey protein". Since there are no relevant standards or common or usual names for such products, Proteus' responsibility is to identify an accurate, non-misleading descriptive name. 21 C.F.R. § 101.3(b)(2)-(3). 9 C.F.R. 317.2(c)(1). Use of the terms "(species) protein" or "concentrated (species) protein" performs such a function in that such terms correctly identify the food product which results from a process in which the protein component of poultry tissue has been extracted. In addition, there is ample precedent at FDA for similar labeling of other vegetable-based protein products produced in a similar fashion. See 21 C.F.R. § 102.22.

Implicit in such a labeling decision is recognition of the fact that the citric acid (or similar food grade, incidental additive) used in the initial processing is appropriately classified as a processing aid.<sup>2</sup> In the proposed formulation, the citric acid fully complies with the FDA definition of a "processing aid" promulgated at 21 C.F.R. § 101.100(a)(3)(ii), in that the citric acid is added to the food for its technical or functional effect in the processing, but is present in the finished poultry product at insignificant levels and does not have a continuing technical or functional effect in that food. See 21 C.F.R. § 101.100(a)(3)(ii)(c).

As described below, the citric acid extracts and purifies the muscle protein and stabilizes the pH in the processing, but is subsequently decreased by a factor of five to seven times during the ultrafiltration process.<sup>3</sup> The citric acid is deliberately decreased by a factor of five to seven

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<sup>1</sup> Twenty-two percent (22%) is the average protein concentration found in poultry muscle.

<sup>2</sup> Citric acid is further a substance affirmed as GRAS. See 21 C.F.R. § 184.1033.

<sup>3</sup> Citric acid as well as salt and water are reduced during the ultrafiltration process because, in contrast to the protein, the small size of the compounds relative to the membrane filter size enables their passage through the filter to the permeate or effluent stream. For example, if we have 100 ml and the starting protein concentration is 22.0 mg/ml and starting citric acid concentration is 7 mg/ml, then we have 2200 mg protein and 700 mg citric acid. Initially, the citric to protein ratio is  $700/2200 = 0.32$ . If we concentrate 5.6 fold times then the protein content stays at 2200 mg

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times during the ultrafiltration process because the citric acid does not provide any additional technical or functional effect in the finished poultry product. Reducing the amount of citric acid in the finished poultry product further removes any sour taste. Filtration, be it ultra- or micro-, is the only method that simultaneously lowers the acid content while concentrating the protein.

### **3. Conditions of Use (Proposed Sec. 170.36(c)(1)(iii))**

This substance will enhance the formulation of a wide variety of further processed poultry items. Given its high protein content, the substance will impart considerable nutritive value to such products. In addition, the substance will have binding characteristics and, as such, might be used in lieu of chemical agents such as phosphates and/or other binding ingredients.

The foods to which the resulting protein can be added are all poultry that have musculature. These include, but are not limited to, chicken, turkey, duck, pheasant and quail. Only protein from identical species will be added, however. Furthermore, while the protein can be added to any poultry muscle, economics dictate that it would be most feasible for higher value products.

Protein is added to muscle in poultry products because the protein has been found to reduce volume shrinkage and increase moistness in the final cooked product while maintaining a high protein level in the finished product. The level used in the final product varies depending upon the final concentration of protein in the protein solution. The higher the protein concentration, the greater the amount that can be applied to the avian musculature, because the solution will contain less water. A detailed analysis of the method of monitoring protein concentration during the ultrafiltration process is provided below in Section 6.

One level of use may be a 10% application rate of an 8% protein solution. A common edible portion of poultry would be 4 oz (112 g) raw poultry which cooks to 3 oz (84 g). The resultant cooked portion would have 0.03 oz (0.90 g) of added protein and 0.003 oz (0.07 g) of citric acid. In "Food Prices to Grow Moderately" (National Restaurant Association, 2004) it was stated that the per capita consumption of edible poultry muscle in the US was 96.2 lbs per year in 2001 and is expected to grow to 105 lbs per year by 2011. Using the higher end of the range, this would translate to an average consumer ingesting approximately 13.5 oz (378 g) of added protein and 1.1 oz (29.4 g) of citric acid per year.

The population expected to consume the protein would be any persons who eat poultry. The protein could be injected into poultry directed for both fresh and frozen markets. Both fresh and frozen injected poultry could be used without additional processing or further processed into value-added products, such as stuffed with a filling, battered alone, or battered and breaded. The

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(doesn't go through the filter) and the citric acid content becomes 125 mg for a ratio of  $125/2200 = 0.057$ . Therefore, we have removed the citric acid from the isolated, concentrated proteins.

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protein solution could also be used as a coating. By using and labeling the protein from the identical species, the consumer would be able to avoid any potential allergen issues.

#### **4. Basis for the GRAS Determination (Proposed Sec. 170.36(c)(1)(iv))**

As explained below in Section 8, the basis for the GRAS determination is “through scientific procedures.”

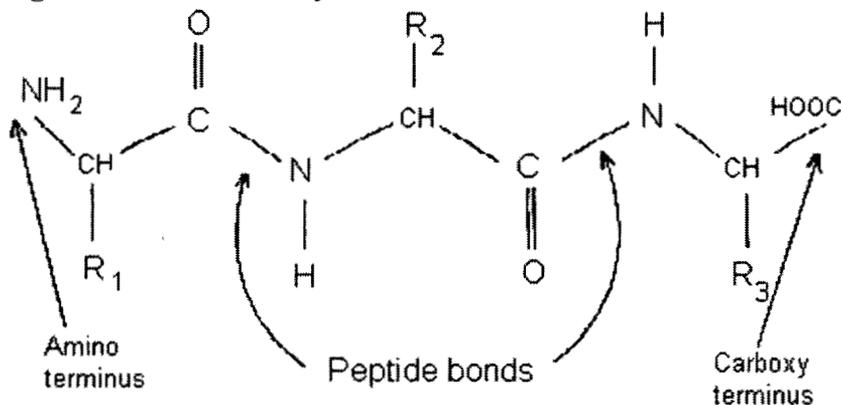
#### **5. Availability of Data and Information (Proposed Sec. 170.36(c)(1)(v))**

The data and information that are the basis for the GRAS determination are available for CFSAN’s review and copying at reasonable times at the address provided above or, upon request, can be sent to CFSAN for review.

#### **6. Identity and Specifications of (Species) Protein or Concentrated (Species) Protein (Proposed Sec. 170.36(c)(2))**

Proteins play an essential role in human nutrition and are utilized by food manufacturers for their functional properties, such as gelatin, water binding ability, fat binding ability, thickeners/viscosity builders, and foaming agents. All proteins follow the same building block format where amino acids are linked together through peptide bonds (**Figure 1**). The linking together of different amino acids in varying sequences is what determines the final structure of the protein and explains why proteins, which basically follow the same construction format, can be totally different in primary, secondary, tertiary, or quaternary structures.

**Figure 1. Basic Primary Protein Structure**

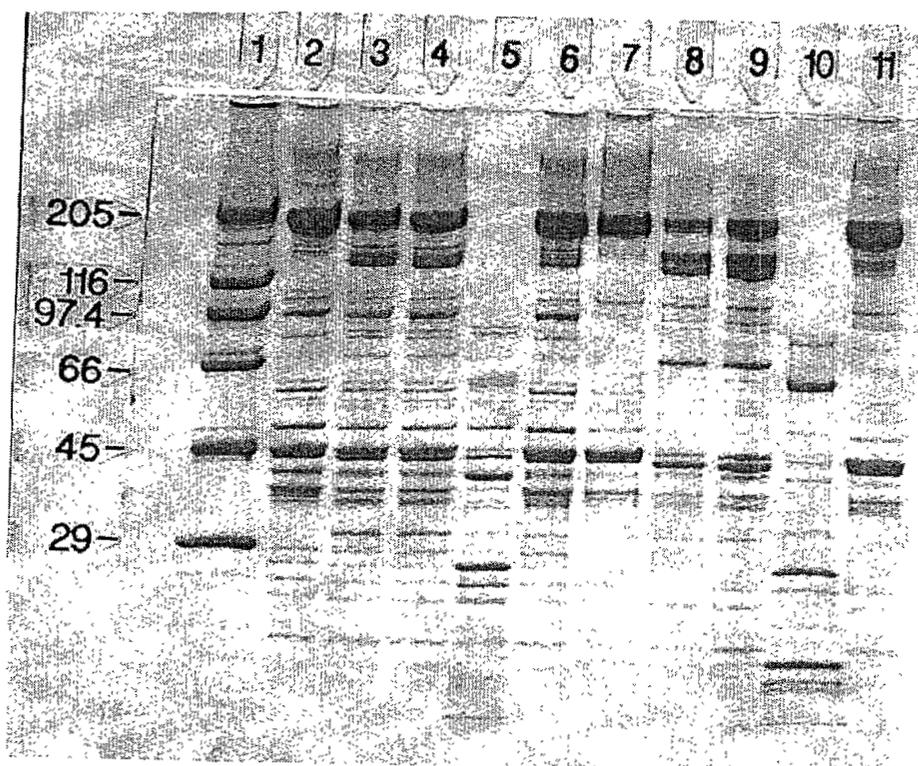


The protein profiles of chicken and protein extracted from chicken using acid solubilization are very similar. **Figure 2** (following page) shows the results from an SDS-PAGE separation of proteins from chicken muscle used as the starting material for the protein extraction

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process.<sup>4</sup> Small peptides removed with the effluent, those that would be found going through an ultrafiltration membrane, are mostly of small size and possibly free amino groups.

**Figure 2 SDS-PAGE (4-20% linear gradient) of chicken breast (light) and thigh & leg (dark) muscle at selected steps in the protein solubilization process.**



An examination and comparison of lanes 2 and 6, and 7 and 11 in **Figure 2** evidences the strong similarity in protein profiles of chicken muscle and protein extracted from chicken breast and thigh and leg muscle using acid solubilization.

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<sup>4</sup>The lane data in Figure 2 are as follows: Lane 1, molecular weight markers; lane 2, chicken breast muscle; lane 3, chicken breast homogenate pH 2.8; lane 4, chicken breast protein supernatant (soluble) from centrifuge (15,000 x g); lane 5, chicken breast supernatant-2 dewatering (10,000 x g); lane 6, chicken breast protein sediment; lane 7, chicken thigh & leg muscle; lane 8, chicken thigh & leg homogenate pH 2.8; lane 9, chicken thigh & leg protein supernatant (soluble) from centrifuge (15,000 x g); lane 10 chicken thigh & leg supernatant-2 dewatering (10,000 x g); lane 11, chicken thigh & leg protein sediment Protein was applied to all lanes at 15 µg/lane.

The protein is extracted using a mild technique which relies on adjustment of pH and salt conditions that perturbs the protein slightly to unfold and expose previously buried hydrophobic areas of the protein. (Kelleher 2000, Kelleher *et al.* 2003). Under low ionic conditions, these unfolded proteins interact to a greater extent with the surrounding water, thus becoming soluble and allowing the removal of insoluble impurities, which is the basis of the process.

The concentrated poultry protein will be manufactured by Proteus in cooperation with its customers at various facilities using the following procedures. (For a pictorial view of the manufacturing processes, we refer you to **Figures 3, 4** (following pages).

The starting material will be in the form of mince or trimmings in either fresh or frozen form. All non-edible tissue will be removed prior to processing including bones. The starting muscle source could originate from any edible muscle source including, but not limited to, chicken, turkey, duck or pheasant.

During the initial processing stage, the starting material will be mixed with cold, potable water to form a slurry. Citric acid (or similar food-grade, acidulant product) will be used for the specific purpose of extracting and purifying the muscle protein and stabilizing the pH of the solution. This is the full extent of the technical or functional effect of the acid. At this point in the manufacturing process, we would estimate, on a percentage basis, that the mixture in question would be comprised of approximately 7.4 percent meat tissue, 91.9 percent water, and 0.7 percent citric acid.

The next step in the process is for these materials to be centrifuged (if the material contains a high content of lipid) or filtered in order to remove or reduce fat and other incidental constituents and materials including contaminants, such as residual bone or skin material, impurities, flavors, odorous compounds and cholesterol. The remaining mixture would consist of protein, water, and citric acid with very low amounts of remaining lipid. After the centrifuge or filtering processes are completed, we anticipate that the resulting material would consist of approximately 1.6 percent protein, 97.7 percent water, and 0.7 percent acid.

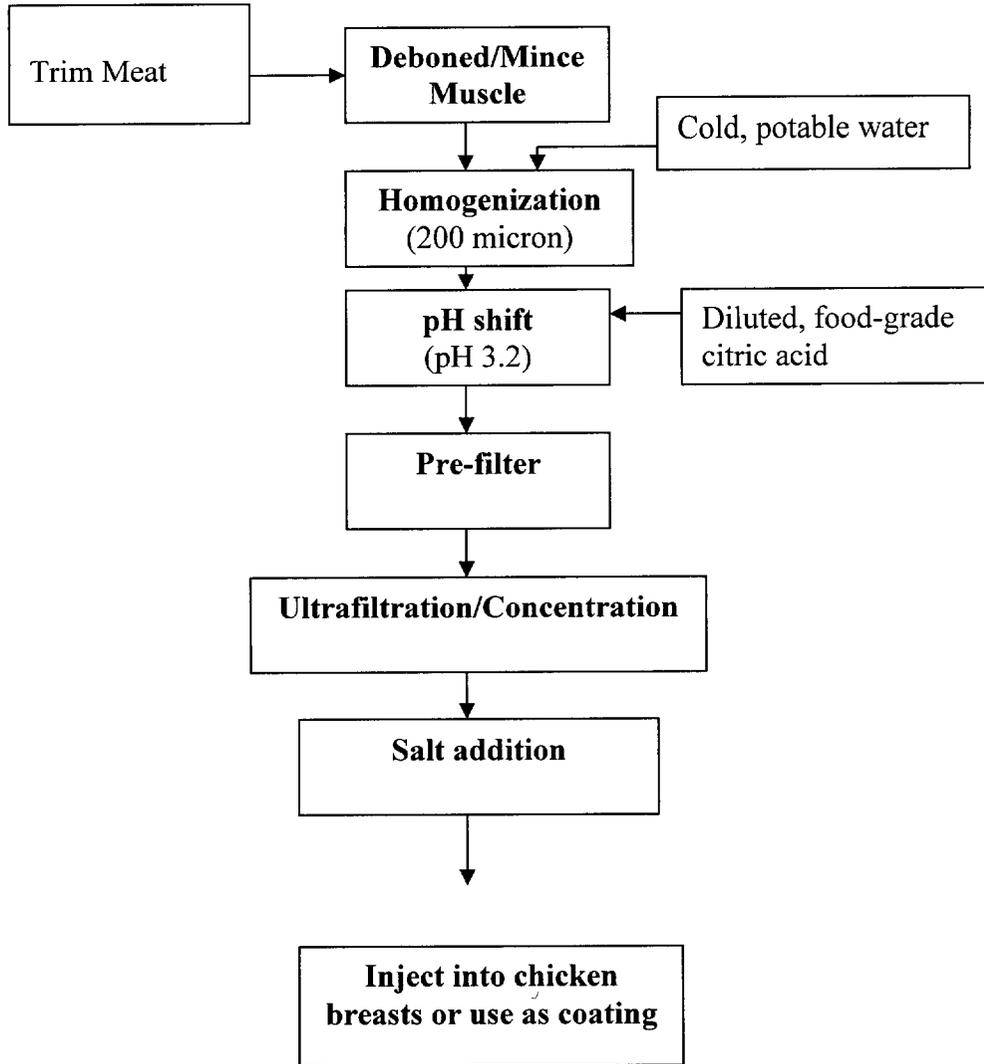
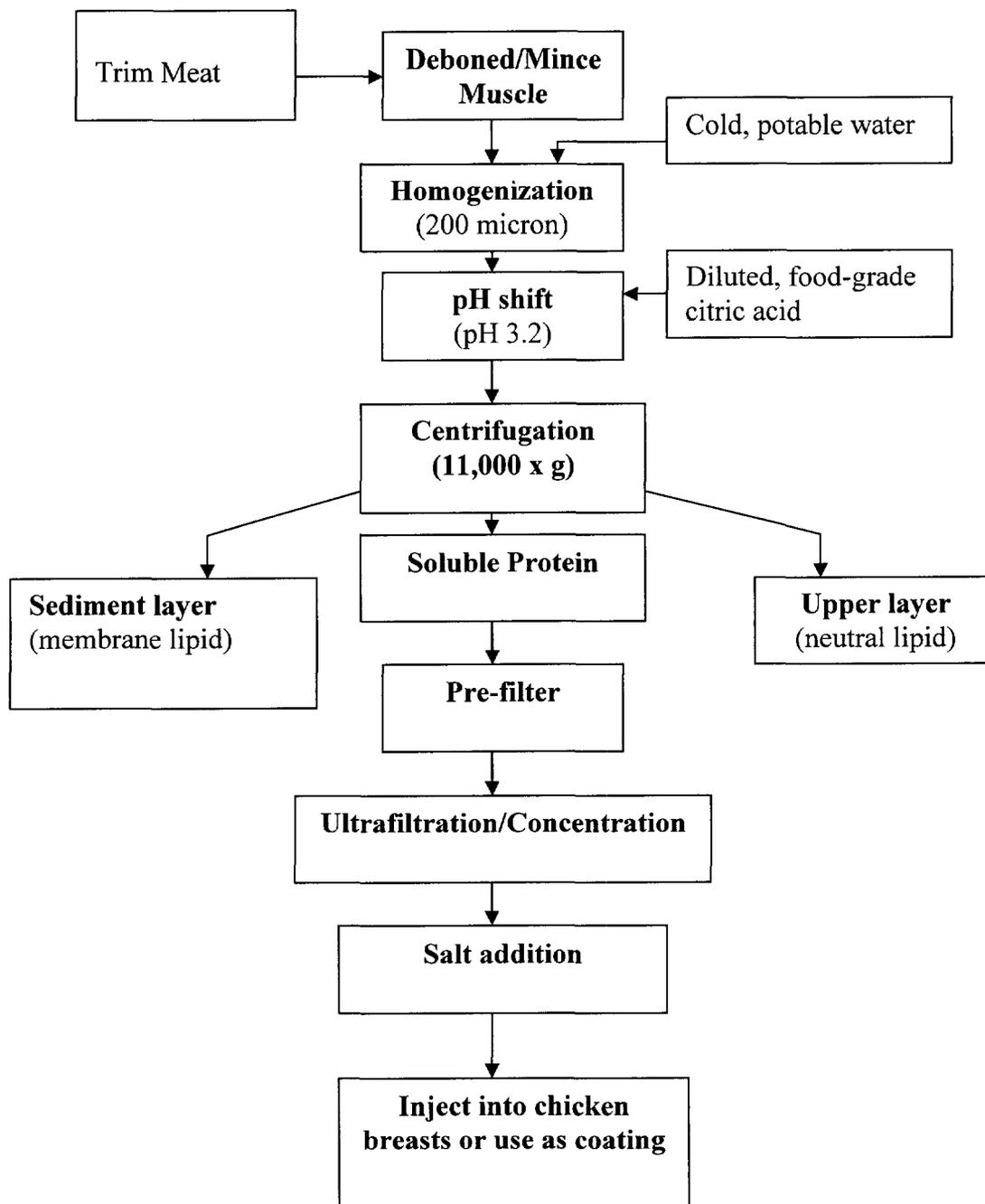


Figure 3. Steps in the acid solubilization protein extraction process.

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**Figure 4.** Steps in the acid solubilization protein extraction process with centrifugation steps.

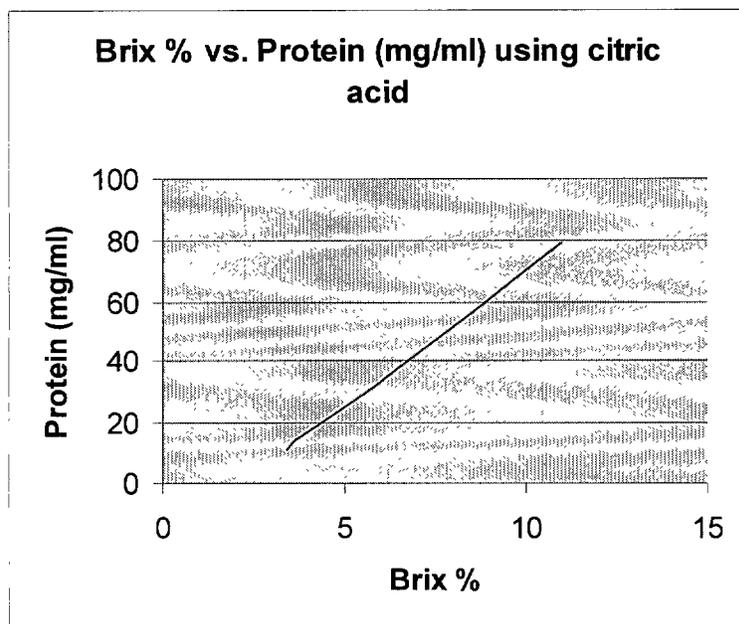
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The next step is to subject the material in question to an ultrafiltration process. The net effect of ultrafiltration is to remove a significant amount of the product's moisture, as well as reduce the citric acid which was added for the sole technical or functional purpose of extracting and purifying the muscle protein and stabilizing the pH during the earlier processing stage. The citric acid is reduced by a factor of five to seven times during the ultrafiltration process. By lowering the citric acid while concentrating the protein, the citric acid to protein ration decreases as ultrafiltration proceeds. The ultrafiltration process also removes salt. The resulting thin-syrup like product is expected to have a protein content between 5-12% and a moisture content range between 88-95%.

Proteus monitors the protein concentration during the ultrafiltration process by utilizing a refractometer which measures soluble solutes (protein being soluble) on a Brix% scale. As the solution concentrates, the Brix % increases. Standard curves (**Figure 5**) relating Brix % to protein concentration can be plotted.

The test was set up to take aliquots of protein solution during different stages of concentration. The protein content is measured, using the Biuret Method<sup>1</sup>, and the Brix %. Plotting the two produces a straight line, which enables Proteus to track the protein using the very simple Brix% test (Brix requires about 1 second to run, whereas protein can take about 1 hour).

**Figure 5. Standard curve for Brix% versus protein concentration.<sup>5</sup>**



<sup>5</sup> Gornall, AG, CS Bardawill, and MM David. *J. Biol. Chem.* 177: 751. 1949.

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Proteus may modify this ultrafiltration process to obtain some variability in the protein content of any finished substance. Using large pore, hollow fiber, ultrafiltration columns, the amount of salt is reduced by a factor equal to the reduction in volume. This results in a salt to protein ratio in the dewatered protein solution that can be lower than in the original tissue. Salt can be added back to the protein solution at a level not to exceed the original tissue salt level. Returning the tissue to the original protein to salt ratio has been shown to improve functionality of the proteins compared to the reduced salt proteins.

The next step is to incorporate the recovered proteins back into tissue of identical species of similar origin. The proteins are added back to the tissue using injection, static soaking, vacuum tumbling, or as a coating. If the proteins are delivered into or on the tissue, then the citric acid level in the final tissue would maximally be between 0.035 and 0.07%, if used, at 5 and 10% application rates, respectively.

The substance is very digestible and is characterized by a fast absorption rate. The extracted proteins have amino acid contents similar to poultry flesh including high levels of aspartic acid, glutamic acid, and lysine (**Figure 6**).

**Figure 6. Amino acid profile of chicken breast muscle and protein extracted from chicken breast muscle using acid solubilization, processed according to US Patent 6,005,073**

<i>Amino acid</i>	<i>Chicken breast protein</i>	<i>Acid solubilized protein from chicken breast</i>
Aspartic acid	12.47	12.63
Threonine*	5.41	5.14
Serine	5.14	4.93
Glutamic acid	19.21	18.63
Proline	3.03	3.85
Glycine	4.54	4.07
Alanine	7.38	7.07
Valine*	4.22	4.50
Methionine*	2.71	2.78
Isoleucine*	3.81	4.07
Leucine*	8.62	8.35
Tyrosine	2.06	2.36
Phenylalanine*	3.39	3.64
Histidine*	3.48	3.21
Lysine*	8.67	8.78
Arginine	5.87	6.00

\* essential amino acids (not including tryptophan)

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**Figure 7** illustrates the effect of the protein extraction process on some endogenous, muscle, tissue components.

**Figure 7. Affect of protein extraction technique on moisture, protein, lipid and phospholipid components using chicken breast and chicken thigh & leg muscle.**

	<i>Moisture (%)</i>	<i>Protein (%)</i>	<i>Lipid (%)</i>	<i>Phospholipid (mg/100g)</i>
<b>Chicken Breast</b>				
Muscle	74.4 ± 0.3	23.6 ± 0.3	1.4 ± 0.1	822.6 ± 0.6
Extracted protein	76.2 ± 0.1	22.1 ± 0.4	0.6 ± 0.0	483.4 ± 34.8
<b>Chicken Thigh &amp; Leg</b>				
Muscle	75.7 ± 0.1	20.3 ± 1.0	5.0 ± 0.0	920.2 ± 42.5
Extracted protein	72.8 ± 0.4	26.8 ± 1.8	1.1 ± 0.0	500.6 ± 104.4

Source: Kelleher (2000). Values are means ± standard deviation on a wet weight basis. Protein was determined using the Biuret method as described by Torten and Whitaker (1969). Lipid was determined using a 1:1 chloroform:methanol extraction solution as described by Lee *et al* (1996). Phospholipid was estimated as phosphorus determined by the dry ashing method of Kovacs (1986) and the assumption that the average MW of phosphatidylcholine was 750 daltons.

Through the process there is a 91.5% recovery of the proteins, 58% reduction in total lipid and a 42% reduction in phospholipids in chicken breast muscle calculated on a dry basis. For chicken thigh and leg muscle there was a 100% recovery of protein, 77% reduction in total lipid, and a 43.4% reduction in phospholipids calculated on a dry basis. Removal of the lipid and phospholipid fractions are believed to improve stability of the extracted proteins. Gandemer (1999) stated that phospholipids are now widely recognized as the main substrates in muscle tissue for lypolysis and oxidation reactions responsible for many of the oxidative off odors and flavors. Research has also pointed to phospholipids as the potential initiator of lipid oxidation reactions (Meynier *et al.*, 1999).

Removal of the lipid (and phospholipid) components can also reduce the concentration of lipid soluble components. Proteus has filed a patent application (US Patent Application # 10/827,646, April 19, 2004) on the reduction of cholesterol when using the soluble proteins in combination with ultrafiltration as described above. Metals, such as iron, are more soluble in oil than in water. Multi- valent cations also seem to be attracted to phospholipids, which tend to have a net negative charge. Removal or reduction of these metal components appears to increase the stability of the final extracted proteins, possibly due to reduced iron ( $\text{Fe}^{+2}$ ) being a known catalyst for oxidation and lipid oxidation reactions as described in the Fenton Equation.  $\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \text{OH}^- + \text{HO}^\cdot$  ( $\text{HO}^\cdot$  is the reactive compound)

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The extracted proteins have also been dried to a stable powder that can be stored without losing their functionality.

We are unaware of any potential human toxicants associated with the extracted proteins.

**7. Self-Limiting Levels of Use (Proposed Sec. 170.36(c)(3))**

We have found that injecting extracted proteins at percentages greater than approximately 18% (w/w; at pH 3.2 using citric acid) may result in a sour taste in the injected poultry product.

**8. Scientific Procedures GRAS Determination (Proposed Sec. 170.36(c)(4))**

We have determined that the “(species) protein” or “concentrated (species) protein” is exempt from premarket approval because such use is GRAS as determined through scientific procedures. That is, there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. Moreover, the information supporting this expert consensus is generally available.

The use of acid solubilized proteins from fish and mammalian muscle tissue, while a relatively new concept, has been covered in much detail in the food scientific literature. Most covered in the literature is the use of acid solubilized proteins for the manufacture of surimi, a crab or seafood analog product, however there are also papers covering beef and chicken.

The safe recovery and use in foods of acid solubilized proteins has been described in the peer reviewed literature by Hultin and Kelleher (1999), Kelleher and Hultin (1999), Hultin and Kelleher (2000), Kelleher (2000), Kelleher and Hultin (2000), Hultin and Kelleher (2001), Cortes-Ruiz *et al.* (2001), Choi and Park (2002), James *et al.* (2002), James and Mireles DeWitt (2002), Mireles DeWitt *et al.* (2002), Undeland *et al.* (2002), Kim *et al.* (2003), Undeland *et al.* (2003) and Kelleher *et al.* (2003). Numerous presentations have also been given on the acceptability of acid solubilized proteins as potential foods, such as those at the Institute of Food Technologists (IFT) Annual Meetings, Pacific Fisheries Technologists (PFT) Annual Meetings, More Efficient Utilization of Fish and Fisheries Products Conference (MEUFPF), Kyoto, Japan, October 2001, and recently at the Trans Atlantic Fisheries Technology (TAFT) Conference held in Reykjavik, Iceland, June 2003.

In these papers and presentations are statements referring to the acid isolated proteins as nutritious, healthful and as a responsible use of by-product proteins for human food use. In Section 3, we described the probable portion intake of the isolated proteins (0.90 g) and citric acid (0.07 g), a value which is quite low when compared against the US RDA of proteins at 63 g protein and the amount of citric acid typically found in orange juice (1%). We are unaware of any potential substances being formed in or on muscle foods due to incorporating acid solubilized, isolated proteins into them.

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We also do not believe that there is any cumulative effect of our isolated proteins in a diet. Both citric acid and isolated proteins have a high degree of water solubility, which makes them less susceptible to accumulation in humans as would lipid soluble ingredients. We have been monitoring research in the field of acid soluble proteins since approximately 1996 and are not aware of any reports of investigations or other information that would be inconsistent with a "(species) protein" or a "concentrated (species) protein" GRAS determination.

Storing or treating muscle proteins in acid as a food has historically been done in the preparation of products such as pickled herring. The acid that appears on most formulas is acetic acid (vinegar). Fish muscle is placed in a solution of acid and salt and marinated for long periods of time after retorted in bottles or cans. We have been unable to find references to the ill effects of consuming acidified, pickled fish products. Proteins of all muscle groups also come in contact with stomach acids as part of the digestion process and this is believed to be a step in improving the nutritional bioavailability of the proteins.

A company licensed by Proteus has been advised to follow the Canadian labeling requirements that require that any protein isolate have a mandatory common name of and be labeled "the name of the source of the protein plus protein" or "the common name of the protein isolate". Following these rules using chicken, FDRB.01.010(3)(a) (Annex, 1, Part 7), "chicken protein" would be appropriate.

There are presently many research groups throughout the United States and the world examining the use of acid solubilized proteins as a food. These research groups are looking at the process for extracting proteins from fish or animals local to them, thereby expanding the regional knowledge.

The use of proteins as a food has been the topic of seminars and demonstrations given at Oregon State University Surimi School (1999-2002), where Dr. Michael Morrissey (Oregon State University), Dr. Jae Park (Oregon State University), and Dr. Stephen Kelleher (University of Massachusetts) led and participated in discussions on the use of acid solubilized proteins as food. All these researchers have continued projects in the area of acid solubilized proteins through U.S. government, SeaGrant, and private funding, the latest being "The use of small pelagics for food applications through the recovery of functional proteins and fish oils" (Dr. Michael Morrissey, Principal Investigator, funded by Oregon SeaGrant and set to begin March 2004).

Other funded research regarding acid solubilized proteins for use as foods is presently taking place in four Scandinavian countries, under a three-year (\$1.3 million USD) Nordic Industry Fund Grant. Researchers in Sweden, Iceland, Denmark, and Norway are studying the isolated proteins from herring by-product in frozen and dried form to be used as seafood analogs, emulsifying agents, and water and fat binding agents. The project manager is Ms. Margret Giersdottir, Icelandic Fisheries Laboratory, in Reykjavik, Iceland. Dr. Christina Mireles DeWitt

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(Oklahoma State University) is also embarking on extracting acid solubilized proteins from beef muscle by-product and catfish frames, both funded by the state of Oklahoma. Dr. Mireles DeWitt has been funded and published in the past on extracting proteins from beef hearts using the low pH extraction process.

It is our belief through discussions with the above mentioned experienced and widely regarded food science researchers that there is a consensus that the acid solubilized extracted proteins are safe as human food.

Sincerely,

Robert G. Hibbert

RGH/crh

cc: Dr. Robert C. Post, FSIS

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**MCDERMOTT, WILL & EMERY**

March 3, 2004

*Received, ky*

*12/3/04*

Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-3835

To Whom It May Concern:

This letter serves to provide the U.S. Food and Drug Administration ("FDA") with notice that our client, Proteus Industries, Inc. ("Proteus"), has concluded that the use of a substance in further processed (or finished) seafood products<sup>1</sup> is exempt from the premarket approval requirements applicable to food additives<sup>2</sup> under the Federal Food, Drug, and Cosmetic Act ("FD&C Act"), 21 U.S.C. § 301 *et seq.*, because such use is generally recognized as safe ("GRAS"). 21 C.F.R. § 170.30.

As explained below, this substance, "(species) protein", or "concentrated (species) protein" depending upon concentration, is acceptable for use in all further processed seafood products, unless specifically precluded by a recipe-type standard of identity. Moreover, to assure compliance with applicable labeling requirements, the presence of any such substance should be identified, in the appropriate order of predominance, in the ingredient statement of any finished seafood product. 21 C.F.R. § 101.4(a)(1). (We are forwarding a copy of this submission to the Office of Nutritional Products, Labeling and Dietary Supplements for its information as well.)

To ensure that the agency will be able, consistent with its established policy, to properly evaluate and respond to this notice within 90 days, the notice tracks the prescribed format and provides a description of the information that the agency considers appropriate to support a GRAS determination as set forth in the Proposed rule, "Substances Generally Recognized as Safe," 62 Fed. Reg. 18937 (April 17, 1997) (proposed 21 C.F.R. § 170.36).

<sup>1</sup> We utilize the terms "further processed" and "finished" interchangeably throughout this notice.

<sup>2</sup> 21 U.S.C. § 348(b)-(f).

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**1. Name and Address of the Notifier (Proposed Sec. 170.36(c)(1)(i))**

As indicated above, the product is produced by Proteus Industries, Inc. which is located at 21 Great Republic Drive, Gloucester, MA 01930. The agency should contact Dr. Stephen Kelleher at this address for further technical information regarding this notification (Telephone: (978) 675-9140 / Facsimile: (978) 675-9194 / E-mail: sdkelleher@proteusindustries.com).

**2. Common or Usual Name of the Substance (Proposed Sec. 170.36(c)(1)(ii))**

An appropriate descriptive name for the substance developed by Proteus for use in finished seafood products is "(species) protein", when the protein concentration is 16% protein or less, or "concentrated (species) protein" if found in concentrated form, a protein concentration greater than 16% protein.<sup>3</sup> Examples would be "Atlantic cod protein" and "concentrated Alaska pollock protein". Since there are no relevant standards or common or usual names for such products, Proteus' responsibility is to identify an accurate, non-misleading descriptive name. 21 C.F.R. § 101.3(b)(2)-(3). Use of the terms "(species) protein" or "concentrated fish protein" performs such a function in that such terms correctly identify the food product which results from a process in which the protein component of fish tissue has been extracted.<sup>4</sup> In addition, there is ample precedent at FDA for similar labeling of other vegetable-based protein products produced in a similar fashion. See 21 C.F.R. § 102.22.

Implicit in such a labeling decision is recognition of the fact that the citric acid (or similar food grade, incidental additive) used in the initial processing is appropriately classified as a processing aid.<sup>5</sup> In the proposed formulation, the citric acid fully complies with the FDA definition of a "processing aid" promulgated at 21 C.F.R. § 101.100(a)(3)(ii), in that the citric acid is added to the food for its technical or functional effect in the processing, but is present in the finished seafood product at insignificant levels and does not have a continuing technical or functional effect in that food. See 21 C.F.R. § 101.100(a)(3)(ii)(c).

As described below, the citric acid extracts and purifies the muscle protein and stabilizes the pH in the processing, but is subsequently decreased by a factor of five to

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<sup>3</sup> Sixteen percent (16%) is the average protein concentration found in fish muscle.

<sup>4</sup> "(species) protein" and "concentrated (species) protein" are sufficiently distinguished from "Whole fish protein concentrate," a food additive with a specific standard of identity. 21 C.F.R. § 172.385.

<sup>5</sup> Citric acid is further a substance affirmed as GRAS. See 21 C.F.R. § 184.1033.

seven times during the ultrafiltration process.<sup>6</sup> The citric acid is deliberately decreased by a factor of five to seven times during the ultrafiltration process because the citric acid does not provide any additional technical or functional effect in the finished seafood product. Reducing the amount of citric acid in the finished seafood product further removes any sour taste. Ultrafiltration is the only method that simultaneously lowers the acid content while concentrating the protein.

**3. Conditions of Use (Proposed Sec. 170.36(c)(1)(iii))**

This substance will enhance the formulation of a wide variety of further processed seafood items. Given its high protein content, the substance will impart considerable nutritive value to such products. In addition, the substance will have binding characteristics and, as such, might be used in lieu of chemical agents such as phosphates and/or other binding ingredients.

The foods to which the resulting protein can be added are all seafood that have musculature. These include, but are not limited to, cod (*Gadus morhua*), flounder (*Hippoglossoides platessoides*), whiting (*Merluccius bilinearis*), Alaska pollock (*Theragra chalcogramma*), scallop (*Argopecten irradians*, *Placopecten magellanicus*), lobster (*Homarus americanus*, *Panulirus spp*) and shrimp (*Pleoticus*). Only protein from identical species will be added, however. Furthermore, while the protein can be added to any seafood muscle, economics dictate that it would be most feasible for higher value seafood products.

Protein is added to muscle in seafood products because the protein has been found to reduce volume shrinkage and increase moistness in the final cooked product while maintaining a high protein level in the finished product. The level used in the final product varies depending upon the final concentration of protein in the protein solution. The higher the protein concentration, the greater the amount that can be applied to the seafood musculature, because the solution will contain less water. A detailed analysis of the method of monitoring protein concentration during the ultrafiltration process is provided below in Section 6.

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<sup>6</sup> Citric acid as well as salt and water are reduced during the ultrafiltration process because, in contrast to the protein, the small size of the compounds relative to the membrane filter size enables their passage through the filter to the permeate or effluent stream. For example, if we have 100 ml and the starting protein concentration is 1.6 mg/ml and starting citric acid concentration is 7 mg/ml, then we have 160 mg protein and 700 mg citric acid. Initially, the citric to protein ratio is  $700/160 = 4.38$ . If we concentrate 5.6 fold times then the protein content stays at 160 mg (doesn't go through the filter) and the citric acid content becomes 125 mg for a ratio of  $125/160 = 0.78$ . Therefore, we have removed the citric acid from the isolated, concentrated proteins.

One limiting factor in applying the protein solution to the seafood musculature is the water that comes in with the proteins. The protein solution will be added in a manner that does not disrupt or cause the natural water to protein ratio to be out of normal range.

One level of use may be a 10% application rate of an 8% protein solution. A common edible portion of fish would be 4 oz (112 g) raw fish which cooks to 3 oz (84 g). The resultant cooked portion would have 0.03 oz (0.90 g) of added protein and 0.003 oz (0.07 g) of citric acid. In "Outlook for Food Prices in 2000" (Clauson, 2000) it was stated that the per capita consumption of edible fish muscle in the US was steady at 14.8 - 15.2 lbs / year. Using the higher end of the range, this would translate to an average consumer ingesting approximately 2.6 oz (73 g) of added protein and 0.2 oz (5.7 g) of citric acid per year.

The population expected to consume the protein would be any persons who eat seafood. The protein could be injected into seafood directed for both fresh and frozen markets. Both fresh and frozen injected seafood could be used without additional processing or further processed into value-added products, such as stuffed with a filling, battered alone, or battered and breaded. By using and labeling the injected protein from the identical species, the consumer would be able to avoid any potential allergen issues.

4. **Basis for the GRAS Determination (Proposed Sec. 170.36(c)(1)(iv))**

As explained below in Section 8, the basis for the GRAS determination is "through scientific procedures."

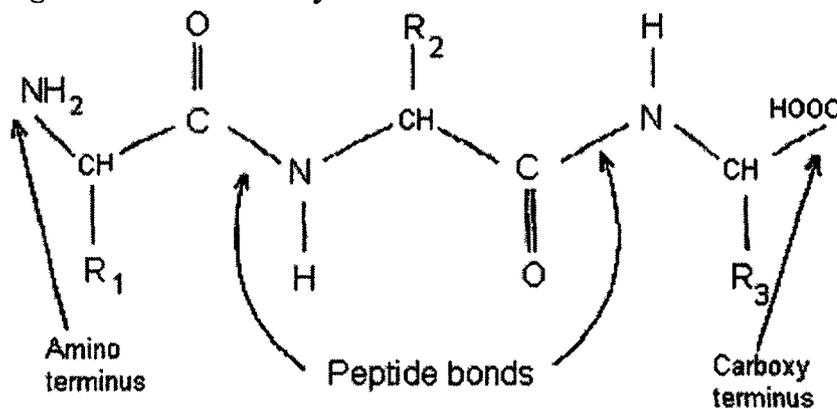
5. **Availability of Data and Information (Proposed Sec. 170.36(c)(1)(v))**

The data and information that are the basis for the GRAS determination are available for FDA's review and copying at reasonable times at the address provided above or, upon request, can be sent to FDA for review.

6. **Identity and Specifications of (Species) Protein or Concentrated (Species) Protein (Proposed Sec. 170.36(c)(2))**

Proteins play an essential role in human nutrition and are utilized by food manufacturers for their functional properties, such as gelatin, water binding ability, fat binding ability, thickeners/viscosity builders, and foaming agents. All proteins follow the same building block format where amino acids are linked together through peptide bonds (**Figure 1**; below, following page). The linking together of different amino acids in varying sequences is what determines the final structure of the protein and explains why proteins, which basically follow the same construction format, can be totally different in primary, secondary, tertiary, or quaternary structures.

**Figure 1. Basic Primary Protein Structure**



The protein profiles of seafood muscle and protein extracted using acid solubilization are very similar. **Figure 2** (below, following page) shows the results from an SDS-PAGE separation of proteins from fish muscle (Atlantic mackerel) used as the starting material for the protein extraction process.<sup>7</sup> Small peptides removed with the effluent, those that would be found going through an ultrafiltration membrane, are mostly of small size and possibly free amino groups.

<sup>7</sup> The lane data in Figure 2 are as follows: Lane 1, molecular weight markers; lane 2, Atlantic mackerel light muscle proteins; lane 3, proteins from homogenate; lane 4, pH 3.0 adjusted homogenate proteins; lane 5, proteins from supernatant layer after centrifugation (10,000 x g); lane 6, proteins from supernatant layer at pH 5.5; and lane 7, proteins from final protein. Protein was applied to all lanes at 15 µg/lane.

**Figure 2** *SDS-PAGE (4-20% linear gradient) of Atlantic mackerel, light muscle at stages of the protein solubilization process.*



An examination and comparison of lanes 2 and 7 in **Figure 2** evidences the strong similarity in protein profiles of fish muscle and protein extracted using acid solubilization.

The protein is extracted using a mild technique which relies on adjustment of pH and salt conditions that perturbs the protein slightly to unfold and expose previously buried hydrophobic areas of the protein. (Kelleher 2000, Kelleher *et al.* 2003). Under low ionic conditions, these unfolded proteins interact to a greater extent with the surrounding water, thus becoming soluble and allowing the removal of insoluble impurities, which is the basis of the process.

The concentrated fish protein will be manufactured by Proteus in cooperation with its customers at various facilities using the following procedures. (For a pictorial view of the manufacturing processes, we refer you to **Figures 3, 4** (below, following pages).

The starting material will be in the form of mince, washed recovered muscle from racks, muscle extracted from collar and belly flap areas in either fresh or frozen form. All non-edible tissue will be removed prior to processing including bones, fins, skin, eyes, and entrails. The starting muscle source could originate from any edible muscle

source including, but not limited to, cod (*Gadus morhua*), flounder (*Hippoglossoides platessoides*), whiting (*Merluccius bilinearis*), Alaska pollock (*Theragra chalcogramma*), scallop (*Argopecten irradians*, *Placopecten magellanicus*), lobster (*Homarus americanus*, *Panulirus spp*) and shrimp (*Pleoticus*).

During the initial processing stage, the starting material will be mixed with cold, potable water to form a slurry. Citric acid (or similar food-grade, acidulant product) will be used for the specific purpose of extracting and purifying the muscle protein and stabilizing the pH of the solution. This is the full extent of the technical or functional effect of the citric acid. At this point in the manufacturing process, we would estimate, on a percentage basis, that the mixture in question would be comprised of approximately 7.4 percent meat tissue, 91.9 percent water, and 0.7 percent citric acid.

The next step in the process is for these materials to be centrifuged (if the material contains a high content of lipid) or filtered in order to remove fat and other incidental constituents and materials including contaminants, such as residual bone or skin material, impurities, flavors, odorous compounds and cholesterol. The remaining mixture would consist of protein, water, and citric acid with very low amounts of remaining lipid. After the centrifuge or filtering processes are completed, we anticipate that the resulting material would consist of approximately 1.6 percent protein, 97.7 percent water, and 0.7 percent acid.

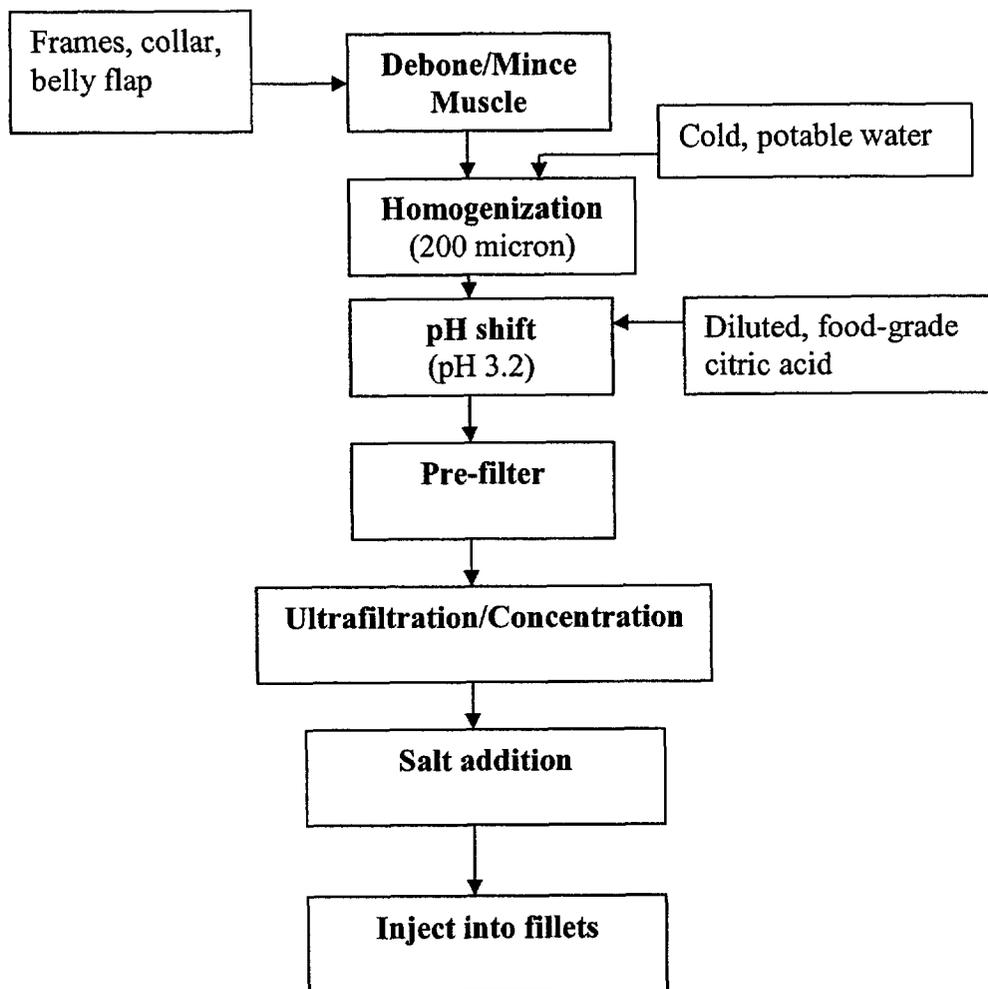
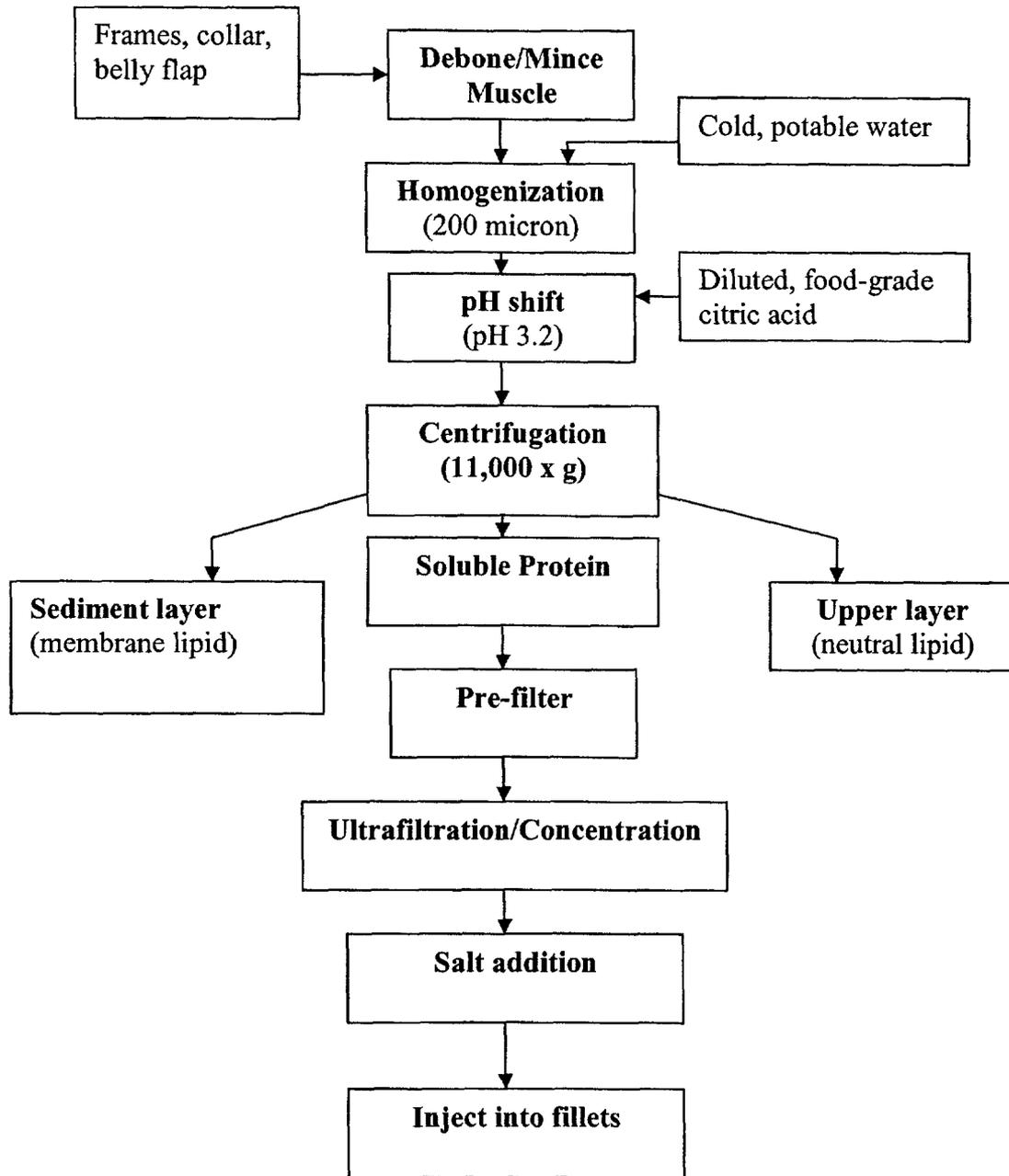


Figure 3. Steps in the acid solubilization protein extraction process.



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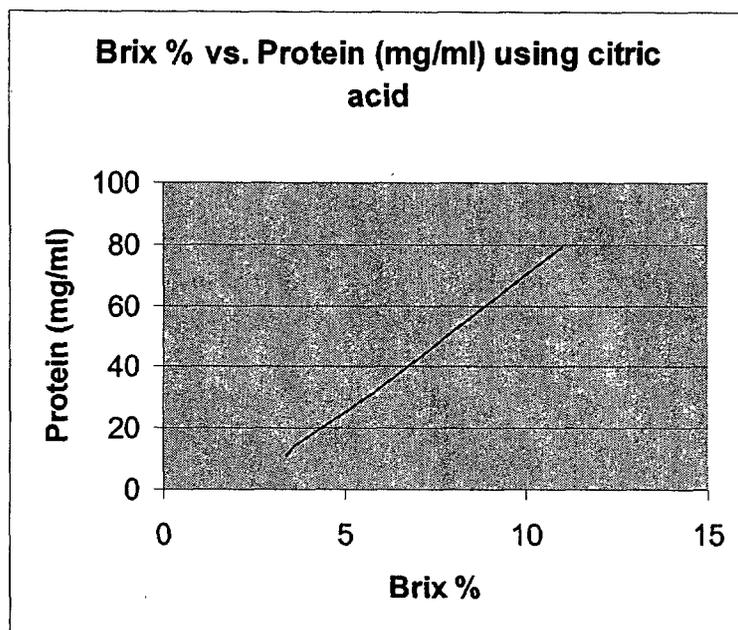
**Figure 4. *Steps in the acid solubilization protein extraction process with centrifugation steps.***

The next step is to subject the material in question to an ultrafiltration process. The net effect of ultrafiltration is to remove a significant amount of the product's moisture, as well as reduce the citric acid which was added for the sole technical or functional purpose of extracting and purifying the muscle protein and stabilizing the pH during the earlier processing stage. The citric acid is reduced by a factor of five to seven times during the ultrafiltration process. By lowering the citric acid while concentrating the protein, the citric acid to protein ration decreases as ultrafiltration proceeds. The ultrafiltration process also removes salt. The resulting thin-syrup like product is expected to have a protein content between 5-12% and a moisture content range between 88-95%.

Proteus monitors the protein concentration during the ultrafiltration process by utilizing a refractometer which measures soluble solutes (protein being soluble) on a Brix% scale. As the solution concentrates, the Brix % increases. Standard curves (Figure 5; below, following page) relating Brix % to protein concentration can be plotted.

The test was set up to take aliquots of protein solution during different stages of concentration. The protein content is measured, using the Biuret Method<sup>1</sup>, and the Brix %. Plotting the two produces a straight line, which enables Proteus to track the protein using the very simple Brix% test (Brix requires about 1 second to run, whereas protein can take about 1 hour).

Figure 5. Standard curve for Brix% versus protein concentration.<sup>8</sup>



Proteus may modify this ultrafiltration process to obtain some variability in the protein content of any finished substance. Using large pore, hollow fiber, ultrafiltration columns, the amount of salt is reduced by a factor equal to the reduction in volume. This results in a salt to protein ratio in the dewatered protein solution that can be lower than in the original tissue. Salt can be added back to the protein solution at a level not to exceed the original tissue salt level. Returning the tissue to the original protein to salt ratio has been shown to improve functionality of the proteins compared to the reduced salt proteins.

The next step is to incorporate the recovered proteins back into tissue of identical species of similar origin. In many cases fish will be filleted, with the muscle tissue in the fish rack being extracted and added back to fillets from the same lot of fish.

The proteins are added back to the tissue using static soaking, vacuum tumbling or needle injection. If the proteins are delivered into the tissue, then the citric acid level in the final tissue would maximally be between 0.035 and 0.07%, if used, at 5 and 10% injection rates, respectively. The substance is very digestible and is characterized by a fast absorption rate. The extracted proteins have amino acid contents similar to fish flesh including high levels of aspartic acid, glutamic acid, and lysine (Figure 6).

<sup>8</sup> Gornall, AG, CS Bardawill, and MM David. *J. Biol. Chem.* 177: 751. 1949.

**Figure 6. Amino acid content of acidified saithe processed according to US Patent 6,005,073**

<i>Amino acid</i>	<i>Total % (w/w)</i>	<i>Amino acid</i>	<i>Total % (w/w)</i>
Ala	4.76	Lys*	9.41
Arg*	7.14	Met*	2.51
Asp	9.47	Phe*	3.26
Glu	13.89	Pro	3.17
Gly	3.61	Ser	3.87
His*	1.80	Thr*	3.98
Ile*	3.44	Tyr*	3.09
Leu*	6.78	Val*	3.69

\* essential amino acids

During the extraction of the proteins, all non-edible tissue are removed, including bones, fins, skin, eyes, and entrails. **Figure 7** (below, following page) illustrates the effect of the protein extraction process on some endogenous, muscle, tissue components.

**Figure 7. Effect of protein extraction technique on protein, lipid and phospholipid components using Atlantic mackerel light muscle.**

	<i>Protein (%)</i>	<i>Lipid (%)</i>	<i>Phospholipid (mg/100g)</i>
Initial muscle	17.8 ± 1.1	9.4 ± 1.1	820 ± 40
Extracted protein	15.1 ± 0.6	0.1 ± 0.1	91 ± 27

Through the process there is a 84% recovery of the proteins, 99% reduction in total lipid and a 89% reduction in phospholipids. Removal of the lipid and phospholipid fractions are believed to improve stability of the extracted proteins. Gandemer (1999) stated that phospholipids are now widely recognized as the main substrates in muscle tissue for lypolysis and oxidation reactions responsible for many of the oxidative off odors and flavors. Research has also pointed to phospholipids as the potential initiator of lipid oxidation reactions (Meynier *et al.*, 1999).

Removal of the lipid (and phospholipid) components can also reduce the concentration of lipid soluble components. Proteus has filed a provisional patent application (US Patent Application # 60/464,617, April 23, 2003) on the reduction of cholesterol when using the soluble proteins in combination with ultrafiltration as described above. Metals, such as iron, are more soluble in oil than in water. Multi-valent cations also seem to be attracted to phospholipids, which tend to have a net negative charge. Removal or reduction of these metal components appears to increase

the stability of the final extracted proteins, possibly due to reduced iron ( $\text{Fe}^{+2}$ ) being a known catalyst for oxidation and lipid oxidation reactions as described in the Fenton Equation.  $\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \text{OH}^- + \text{HO}^\cdot$  ( $\text{HO}^\cdot$  is the reactive compound). The extracted proteins have also been dried to a stable powder that can be stored without losing their functionality.

We are unaware of any potential human toxicants associated with the extracted proteins.

**7. Self-Limiting Levels of Use (Proposed Sec. 170.36(c)(3))**

We have found that injecting extracted proteins at percentages greater than approximately 18% (w/w; at pH 3.2 using citric acid) may result in a sour taste in the injected seafood product.

**8. Scientific Procedures GRAS Determination (Proposed Sec. 170.36(c)(4))**

We have determined that the "(species) protein" or "concentrated (species) protein" is exempt from premarket approval because such use is GRAS as determined through scientific procedures. That is, there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. Moreover, the information supporting this expert consensus is generally available.

The use of acid solubilized proteins from fish and mammalian muscle tissue, while a relatively new concept, has been covered in much detail in the food scientific literature. Most covered in the literature is the use of acid solubilized proteins for the manufacture of surimi, a crab or seafood analog product.

The safe recovery and use in foods of acid solubilized proteins has been described in the peer reviewed literature by Hultin and Kelleher (1999), Kelleher and Hultin (1999), Hultin and Kelleher (2000), Kelleher (2000), Kelleher and Hultin (2000), Hultin and Kelleher (2001), Cortes-Ruiz *et al.* (2001), Choi and Park (2002), James *et al.* (2002), James and Mireles DeWitt (2002), Mireles DeWitt *et al.* (2002), Undeland *et al.* (2002), Kim *et al.* (2003), Undeland *et al.* (2003) and Kelleher *et al.* (2003). Numerous presentations have also been given on the acceptability of acid solubilized proteins as potential foods, such as those at the Institute of Food Technologists (IFT) Annual Meetings, Pacific Fisheries Technologists (PFT) Annual Meetings, More Efficient Utilization of Fish and Fisheries Products Conference (MEUFPF), Kyoto, Japan, October 2001, and recently at the Trans Atlantic Fisheries Technology (TAFT) Conference held in Reykjavik, Iceland, June 2003.

In these papers and presentations are statements referring to the acid isolated proteins as nutritious, healthful and as a responsible use of by-product proteins (fishmeal) for human food use. In Section 3, we described the probable portion intake of the

isolated proteins (0.90 g) and citric acid (0.07 g), a value which is quite low when compared against the US RDA of proteins at 63 g protein and the amount of citric acid typically found in orange juice (1%). We are unaware of any potential substances being formed in or on muscle foods due to incorporating acid solubilized, isolated proteins into them.

We also do not believe that there is any cumulative effect of our isolated proteins in a diet. Both citric acid and isolated proteins have a high degree of water solubility, which makes them less susceptible to accumulation in humans as would lipid soluble ingredients. We have been monitoring research in the field of acid soluble proteins since approximately 1996 and are not aware of any reports of investigations or other information that would be inconsistent with a "(species) protein" or a "concentrated (species) protein" GRAS determination.

Storing or treating muscle proteins in acid as a food has historically been done in the preparation of products such as pickled herring. The acid that appears on most formulas is acetic acid (vinegar). Fish muscle is placed in a solution of acid and salt and marinated for long periods of time after retorted in bottles or cans. We have been unable to find references to the ill effects of consuming acidified, pickled fish products. Proteins of all muscle groups also come in contact with stomach acids as part of the digestion process and this is believed to be a step in improving the nutritional bioavailability of the proteins.

A company contracted by Proteus has been advised to follow the Canadian labeling requirements that require that any protein isolate have a mandatory common name of and be labeled "the name of the source of the protein plus protein" or "the common name of the protein isolate". Following these rules using cod, FDRB.01.010(3)(a) (Annex, 1, Part 7), "cod protein" would be appropriate.

There are presently many research groups throughout the United States and the world examining the use of acid solubilized proteins as a food. These research groups are looking at the process for extracting proteins from fish or animals local to them, thereby expanding the knowledge to the local catch.

The use of proteins as a food has been the topic of seminars and demonstrations given at Oregon State University Surimi School (1999-2002), where Dr. Michael Morrissey (Oregon State University), Dr. Jae Park (Oregon State University), Dr. Tyre Lanier (North Carolina State University), Dr. Herbert Hultin (University of Massachusetts) and Dr. Stephen Kelleher (University of Massachusetts) led and participated in discussions on the use of acid solubilized proteins as food. All these researchers have continued projects in the area of acid solubilized proteins through U.S. government, SeaGrant, and private funding, the latest being "The use of small pelagics for food applications through the recovery of functional proteins and fish oils" (Dr. Michael Morrissey, Principal Investigator, funded by Oregon SeaGrant and set to begin March 2004).

To Whom It May Concern,

March 3, 2004

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Other funded research regarding acid solubilized proteins for use as foods is presently taking place in four Scandinavian countries, under a three-year (\$1.3 million USD) Nordic Industry Fund Grant. Researchers in Sweden, Iceland, Denmark, and Norway are studying the isolated proteins from herring waste in frozen and dried form to be used as seafood analogs, emulsifying agents, and water and fat binding agents. The project manager is Ms. Margret Giersdottir, Icelandic Fisheries Laboratory, in Reykjavik, Iceland. Dr. Christina Mireles DeWitt (Oklahoma State University) is also embarking on extracting acid solubilized proteins from beef muscle by-product and catfish frames, both funded by the state of Oklahoma. Dr. Mireles DeWitt has been funded and published in the past on extracting proteins from beef hearts using the low pH extraction process.

It is our belief through discussions with the above mentioned experienced and widely regarded food science researchers that there is a consensus that the acid solubilized extracted proteins are safe as human food.

Sincerely,

Robert G. Hibbert  
Counsel for Proteus Industries, Inc.

WDC99 887883-1.067559.0010

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Submission End

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