PROGRESS REPORT
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ANALYSIS OF SEPARABLE FRACTIONS OF PROTEINS FROM SELECTED ANIMAL BY-PRODUCT MATERIALS

Principal Investigator(s): James C. Acton, Stender Professor Emeritus
jcacton@*clemson.edu*
Department of Food Science & Human Nutrition
P.O. Box 340316
Clemson University
Clemson, SC 29634-0316
(864) 656-5681/(864) 656-0331 FAX

Collaborators: Ashley B. Bodine, Professor and Chair
Department of Animal & Veterinary Science
P.O. Box 340361
Clemson University
Clemson, SC 29634
(864) 656-3120/(864) 656-3131 FAX

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Duration of Project: 12 months + Extension

Objective(s):

The overall objectives were to quantify separable protein fractions (via solvent extraction) from by-products, determine fraction characteristics (electrophoresis for subunits and estimated molecular weights) and evaluate useful functional properties of the protein fractions.

Project Overview:

Protein of all materials is generally classified into type based on solubility in several solvent systems. Classic solvent groups include water soluble (albumins), salt-soluble in dilute salt solution (globulins), alcohol soluble in 70% ethanol (prolamins), and alkali soluble in 0.1N sodium hydroxide. In the case of animal by-products entering the rendering stream, all fractions exist co-mingled from the various materials (bone, hair/feathers, skin, organs, tissues) being processed. Simple extraction processes using water and/or salt solution would appear feasible to separate and isolate potentially useful proteins since the aqueous phase could be added back to the "cook" stream during processing after protein separation.

Any economic benefit would be dependent on the functional behavior of the proteins, for example, gelation, emulsifying, water-binding, adhesiveness and foaming abilities.

Co-Products. Five rendered products categorized as "meat" from 1 processor, "meat meal" from 4 processors, "meat and bone meal" from 4 processors, "poultry meal" from 2 processors, and "meat and poultry" from 1 processor were evaluated by category. These products were obtained from Dr. Xiuping Jiang's laboratory samples collected in another funded FPRF study. Representative category samples analyzed for this study are given in **Table 1** along with "as is" properties.

TABLE 1. Moisture, protein, water activity and pH of five categories of rendered products.

Co-Product Category	Moisture[1] (%)	Protein[2] (%) (N x 6.25)	Water Activity[3] (a_w)	pH[4] (in H_2O)
Meat	4.33	65.56	0.33	6.08
Meat Meal	1.58	56.66	0.32	6.36
Meat + Bone Meal	5.29	61.81	0.37	6.42
Poultry Meal	2.55	67.41	0.36	6.51
Meat + Poultry	5.00	53.56	0.26	6.00

[1]AOAC (1995) 39.1.02B(a); [2]AOAC (1995) 39.1.15 Alternative II; [3]Rotronic D2102 Water Activity Systems (Rotronic Instruments, Huntington, NY); [4]1% solution at room temperature.

As meal products, all were low in moisture content and as a result, also had low water activities (aw) indicating high stability against potential microbial outgrowth. The products also have a fairly neutral pH.

Extractable Protein Fractions. Separate samples of products in each category from Table 1 were extracted with three solvents: (a) water, (b) 0.6M NaCl, and (c) 0.1N NaOH. A 10:1 (v/w) initial sample extraction was followed by a second 10:1 (v/w) extraction on the residue, with

supernatants after centrifugation (8XXXg) filtered and combined. Protein in the filtrates was determined by Biuret reaction and the percent of total protein extracted was calculated as given in **Table 2**.

TABLE 2. Extractable protein fractions (% of total protein) in five categories of rendered products.

Co-Product Category	Water-Soluble (%)	Salt-Soluble (%)	Alkali-Soluble (%)
Meat	6.29	7.77	62.84
Meat Meal	6.26	6.23	24.29
Meat + Bone Meal	8.29	12.52	43.30
Poultry Meal	6.27	14.06	23.98
Meat + Poultry	9.81	8.01	48.71

The water extractable protein fraction was the lowest quantity in most cases followed closely by the quantity of salt-soluble protein. The salt-soluble fraction, using our extraction procedure, would contain most of the same proteins that are also present in the water-soluble fraction. A much higher fraction quantity of salt-soluble protein was expected due to usual presence of some fleshy tissues that would contain sarcoplasmic and myofibrillar type proteins. However, significant denaturation is evident due to the low extractability found in all samples.

Alkali-soluble protein content ranged from approximately 24% (Meat Meal and Poultry Meal) to a high of approximately 63% (Meat). Many types of proteins are soluble in alkali (NaOH) and it is likely some of the gelatinous proteins are included in this fraction. Extractions were conducted at 3-4^{o}C, thus the gelatinous proteins likely would not be present in the water- and salt-soluble fractions. Since the alkali fraction yield was, at a minimum, a fourth of the protein content (based on Kjeldahl N) upwards to 40-63%, it would be useful to explore this fraction further for preheat treated co-products.

Emulsifying Capacity and Gelation Ability. Each fraction was analyzed for emulsifying capacity which consists of blending an aliquot of extract placed in a Waring Blender jar with corn oil that is metered into the solution vortex as it is being blended. As the mixture becomes viscous, the endpoint for emulsifying capacity (ml oil emulsified/100 mg protein) is detected as an abrupt drop in mix viscosity. For gelation ability, 5 mL of original extract fractions were separately heated to 70^{o}C and held for 30 min after which they are allowed to cool to ambient temperature and the tube inverted. If a gel is present, dilutions are made until no continuous gel is observed. The results of these tests are given in **Table 3**.

TABLE 3. Emulsifying capacity (ml/100 mg protein) and gelation ability for protein fractions in five categories of rendered products.

Product Category	Water-Soluble (%)		Salt-Soluble (%)		Alkali-Soluble (%)	
	EC	Gelation	EC	Gelation	EC	Gelation

Meat	45	negative	38	negative	31	negative
Meat Meal	60	negative	60	negative	14	negative
Meat + Bone Meal	42	negative	27	negative	10	negative
Poultry Meal	50	negative	21	negative	13	negative
Meat + Poultry	32	negative	48	negative	10	negative

Emulsifying capacity data appear very positive when compared with data from prior studies using myofibrillar or salt-soluble protein extracts of muscle tissues. However, the capacity values were highly dependent on the procedure rather than on the protein's function since all visual "collapse" points at "maximum oil addition" ranged from 21 ml to 34 ml, with 11 of the 15 comparisons in a narrow range of 23 to 26 ml. Thus, the actual collapse point occurred at an oil/water phase volume of 0.48-0.50 (i.e., near 50% oil-in-water). A more appropriate evaluation would be emulsion stability of formed emulsions of varying phase volumes rather than the "blend to break". The true stabilizing ability of the proteins could be determined.

No gelling ability was evident for any protein fraction. The extracts were not concentrated to increase protein content. Therefore if any fraction does have gelling ability, it is above the protein concentration found by extraction with this sample-to-solvent ratio.

Electrophoresis of Extracted Protein Fractions. Each fraction was analyzed by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) using Bio-Rad's Ready Gel Tris-HCl precast mini gels (4-20%) following standard sample preparation, application and separation in a Mini-PROTEAN II cell (Bio-Rad Laboratories, Inc., Hercules, CA). Representative gels are shown in **Figures 1 and 2** along with reference standards for molecular weight (1 kd = 1,000) comparisons.

In **Figure 1**, all water-extractable and alkali-extractable protein lanes samples applied in Lanes L3-L6 and L9-L12, respectively, for Poultry Meal, Meat Meal, Meat + Bone Meal, and Meat samples show a generalized smear down the lane of decreasing molecular weight (lanes are vertical). Water extracted fractions contained less protein and very little stain was adsorbed by protein so the lanes appear clear except for very light smearing, meaning no major sub-fraction proteins of significant concentration. Alkali (NaOH) extracted proteins contained the highest concentrations of protein (**Table 2**), and while smearing occurred, intense stain of protein can be seen at the starting lane at the top

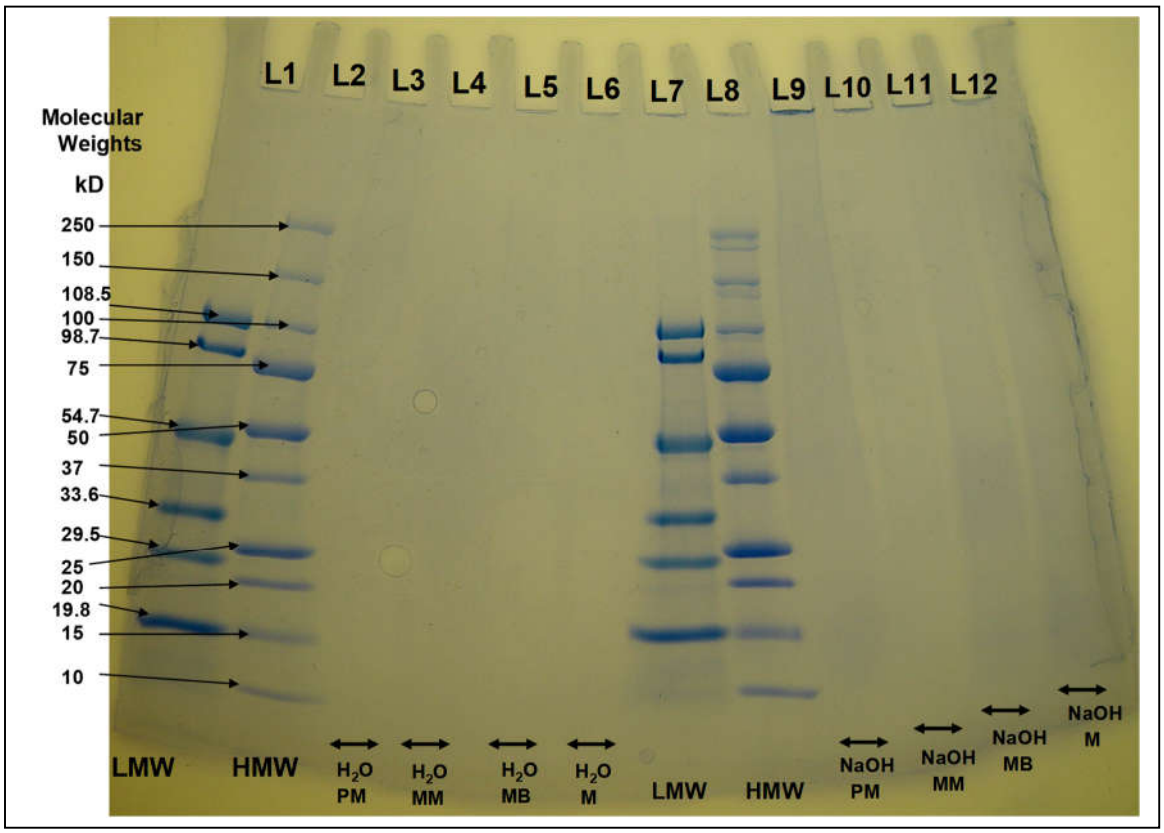

Figure 1. SDS-PAGE patterns of low and high molecular weight standards (LMM and HMM, respectively) and of water (H_2O) and NaOH extracts from Poultry Meal (PM), Meat Meal (MM), Meat + Bone Meal (MB) and Meat (M). Molecular weights are indicated for the standards in kilodaltons (x 1000). Lanes L3 to L6 and L9 toL12 are sample lanes.

of the gels. Similar stain appears at the top lanes (L1-L4) for the salt (NaCl) extracted proteins for these same rendered product samples (**Figure 2**). These results for the heat treated products means that, if present in an undenatured or renatured form, their concentration is extremely low and not identifiable, and, that the heavy stain at the top of the lane indicates they were aggregated (covalently bonded chains) due to heating and are of very high molecular weight. These results also suggest that use of any extracted protein fraction should be used without further separation and treated as "one" protein source for any function or utilization or further application.

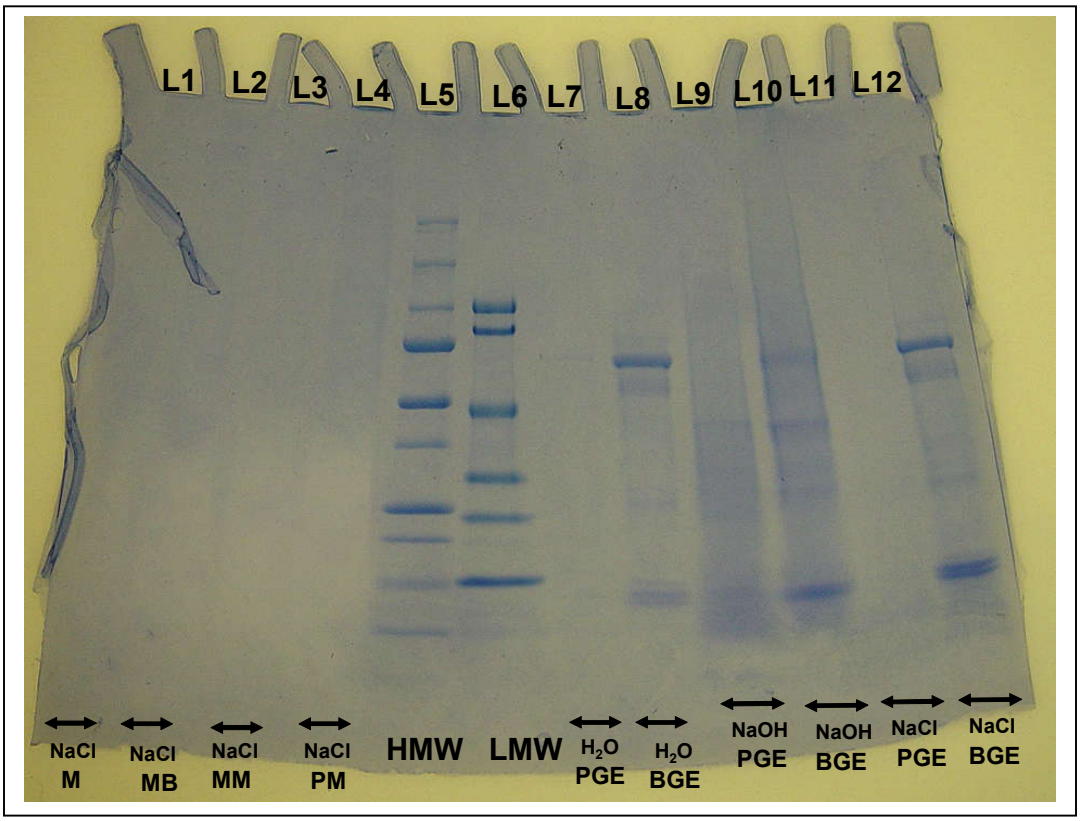

Figure 2. SDS-PAGE patterns of low and high molecular weight standards (LMM and HMM, respectively), of NaCl extracts from Meat (M), Meat + Bone Meal (MB), Meat Meal (MM), Poultry Meal (PM), and of water (H_2O), NaOH and NaCl extracts from raw samples of Poultry Guts (PGE) and Beef Guts (BG). Molecular weights are indicated for the standards in kilodaltons (x 1000). Lanes L1 to L4 and L7 toL12 are sample lanes.

Poultry and Beef Gut samples (raw) (from Dr. Annel Greene's project) were also extracted with the same solvents and examined to confirm a difference between preheat treated rendered products and a raw material entering the rendering process stream. In **Figure 2**, gel patterns of water, salt, alkali and salt extracts of the Beef Gut samples show several small sub-unit proteins and major proteins at molecular weights of approximately 60 kd, 50 kd, 37 kd, and 18 kd in their respective lanes (L8, L10 and L12). Faint, smaller concentration bands of protein are shown for Poultry Guts in the water and alkali extracts (L7 and L9, respectively) at weights of approximately 60 kd, 37 kd, and 18 kd. These results confirm that native, raw material entering the rendering stream for product treatment do contain significant proteins within the various potential solvent fractions. Viewing the gels for the gut sample extracts (**Figure 2**) also indicates that in the raw state, extraction with NaCl is potentially a better solvent than water or alkali. Further work should be oriented to exploring extractions of raw materials if microbial contaminants can be eliminated prior to extraction.

Impacts and Significance:

From the five categories of co-products manufactured via heating and dehydration, only proteins of the alkali-soluble fraction generally provided a yield (based on total protein content) that indicated extraction might be of economic value. Emulsification and gelation abilities of water-soluble, salt-soluble, and alkali-soluble protein fractions were evaluated. Based on the analytical methods used for evaluation, no fraction possessed these functional properties. Protein denaturation is obviously significant in processing of the co-product materials from the raw to finished state as indicated in the electrophoresis analysis of heated versus raw type materials.

Publications:

None at present. This study will be submitted as a publication contribution at the 2006 International Congress of Meat Science and Technology to be held August 13-18.

A modified FPRF pre-proposal based on the current study titled "Yield and Functional Analyses of Protein Fractions of Bone Residues from Poultry Mechanical Deboning Operations" was submitted to the International Poultry and Egg Association (formerly US Poultry & Egg) in April 2004. The proposal was not of interest at that time.

Future Work:

1. Evaluate the extractability of the protein fractions from raw materials prior to thermal treatment if a non-thermal process can be used to destroy the microflora accompanying these materials. Significant yields and functional activity retention would be expected that would provide economic benefit. Solvents used in extraction, particularly water and salt solution, could be returned to the cooker after protein precipitation via ionic strength or pH alteration. Neutralization of an alkali solvent is also possible.
2. Since a relatively high yield of the alkali-soluble protein fraction was obtained from pre-heated co-product samples as utilized in this study, the fraction could be neutralized, protein precipitated and recovered and then evaluated for functional properties. Changing the environmental solvent surrounding the recovered protein may restore some functionality. Suspended protein can have excellent emulsifying ability and does not have to be completely soluble.
3. Emulsion capacity testing was found to be of no value in this study for pre-heat treated co-products. In future studies, emulsion stability from emulsions formed with differing phase volume ratios of oil-in-water preparations would be more appropriate.

Acknowledgments:

The investigators thank Dr. Xiuping Jiang for providing the samples in her collection of co-products from her FPRF funded study. The investigators also thank Dr. Annel Greene for personal assistance in electrophoretic examination of the extracts and providing samples of raw materials from her project.

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