The Functional Requirements of Proteins for Foods[1]

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ABSTRACT

As a growing amount of research attention has been diverted, for a number of reasons, from the traditional protein foods to the so-called unconventional food proteins, an awareness has increased of the need to understand the functional properties of these proteins. Some empirical functionality tests have been devised, but it is submitted that many of these could yield misleading information, inasmuch as they often ignore or even run counter to the environmental interactions to which proteins are exposed in food systems. Some examples are given of the influence of the ionic environment upon one basic functional property of proteins, their solubility in aqueous solution.

Proteins are not foods; they are food components or food ingredients. While proteins per se are recognized as being essential dietary nutrients, the common protein foods (meat, fish, eggs and dairy products) owe their widespread appeal to the gastronomic pleasure they afford rather than to their nutritional value. In these foods, the proteins are structural components that contribute specific functional properties directly associated with their popularity as foods.

The animal protein foods are expensive both in terms of land requirements and market price. In recognition of the worldwide need for more dietary protein, particularly for low-income groups, there have been extensive efforts to develop low-cost protein foods. Because of the realities of marketing, many of these are frank imitations of the natural animal-derived protein products. Therefore, it becomes important to simulate the functional properties of the animal proteins that make their products so attractive to the consumers. The ultimate objective is to be able to create any type of protein food system from low-cost raw materials.

This is quite similar to the situation that existed in the margarine and shortening industries 25 years ago. Their objective then was to attain a maximum of flexibility in the selection of raw materials. That objective was attained primarily through investigations of the fundamental physical and chemical properties of glycerides and their relationship to functional and performance properties. Although the systems, and therefore the problems, are much more complex in protein foods, the big accomplishments once again will derive from relating the chemical and physical properties of proteins to function and performance.

There are a multitude of sources of low-cost proteins. These include the major oilseeds, fish and the various types of single-cell proteins. As such, they are only raw materials and the challenge of technology is to convert them into useful food ingredients. Their successful use in foods that can be made and sold at a profit will depend upon their functional properties rather than upon their nutritional qualifications. The proteins from each of these raw materials should be considered to be unique and possessing inherent functional properties not necessarily similar to those from any of the others. Therefore, a systematic investigation of the functional properties of the proteins from each of these sources should be undertaken.

The range of desirable and attractive functional properties that should be looked for is almost as broad as the range of foods themselves. For example, if one is considering producing a beverage, two desirable functional properties would be solubility and suitable viscosity. For bread, the need is for a protein that is compatible with gluten. For various meat systems, desirable qualities would include water-binding, emulsifying properties and the ability to be formed into fibers. For other purposes, the properties of gel formation, whippability, adhesiveness and thickening might be considered beneficial.

This is a rather substantial array of functional properties. To evaluate each of the low-cost protein sources for all of the possible functional uses would be a rather large undertaking. The problem is compounded by the fact that for the most part, there are no generally accepted tests for evaluating the several functional properties, and the tests that are available are quite empirical. Further, it is our opinion that in the absence of certain basic information, the empirical functionality tests could yield misleading information.

We believe that an investigation of the functional properties of any protein can be made more efficient if a systematic study is first made of the solubility properties of that protein in a variety of ionic environments. Such information can give valuable clues as to potential uses for the protein, as well as indicate the inapplicability of this protein for some other uses.

Quite a bit of research was done 25-30 years ago on the solubility profiles of soybean, cottonseed and peanut proteins (1,2). This information proved valuable in devising methods for the preparation of soybean and peanut isolates and more recently, of soybean protein concentrates. Solubility data have also led the investigators in the USDA Laboratory in New Orleans in the development of two protein isolates and a protein concentrate from cottonseed (3,4). Such data also gave the first clue to the potential use for one of the isolates in carbonated beverages.

Beyond this, there appears to be little evidence that the technique of the nitrogen solubility profile is being used to optimum advantage as a guide to functionality. Using some data largely from our own laboratories, we will try to point out some of the ways that the solubility profile can be useful.

Extraction of proteins for solubility profile analysis in our laboratories is done at room temperature, with magnetic stirring for at least 30 min, using a solvent to meal ratio of 20:1 (v/w). The pH of extraction is obtained by addition of 0.5 N NaOH or 0.5 N HCl; the pH is rechecked and readjusted during extraction. Sample size is 2.0 g, and the volume of the solution is brought to 40 ml after the final pH adjustment. After centrifugation at 4300 x g for 20 min, the supernatant extract is filtered through Whatman No. 1 filter paper. A 20 ml aliquot of the extract is taken for nitrogen analysis. The data shown as nitrogen solubility include all nitrogen in aqueous solution, including that which remains absorbed on the meal. For practical recovery information, calculations are based only on the nitrogen in the supernatant, separable extract.

In Figure 1 are shown the nitrogen solubility profiles of glandless cottonseed meal, coconut meal and sunflower

[1]One of 21 papers presented at the Symposium, "Oilseed Processors Challenged by World Protein Need," ISF-AOCS World Congress, Chicago, September 1970.

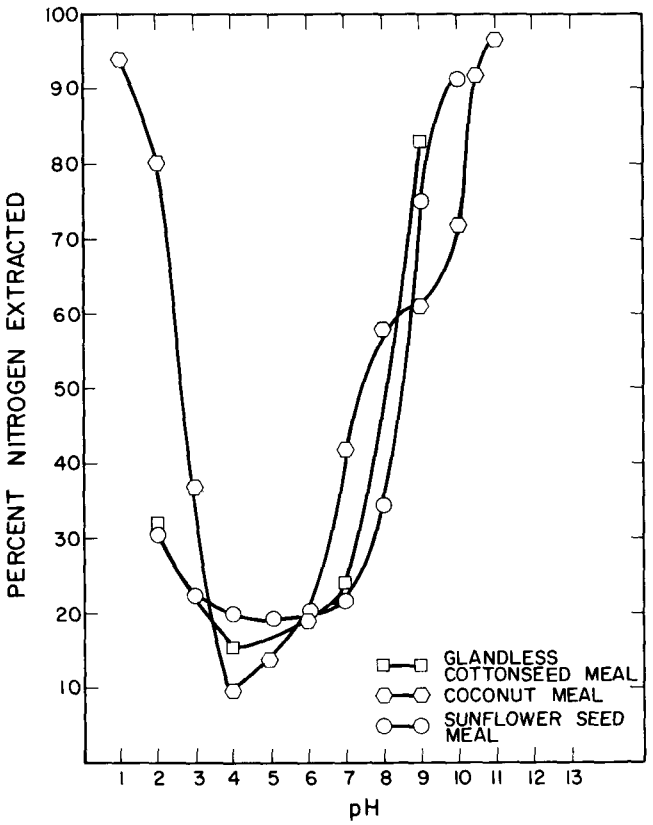

FIG. 1. Protein solubility profiles of several oilseed meals.

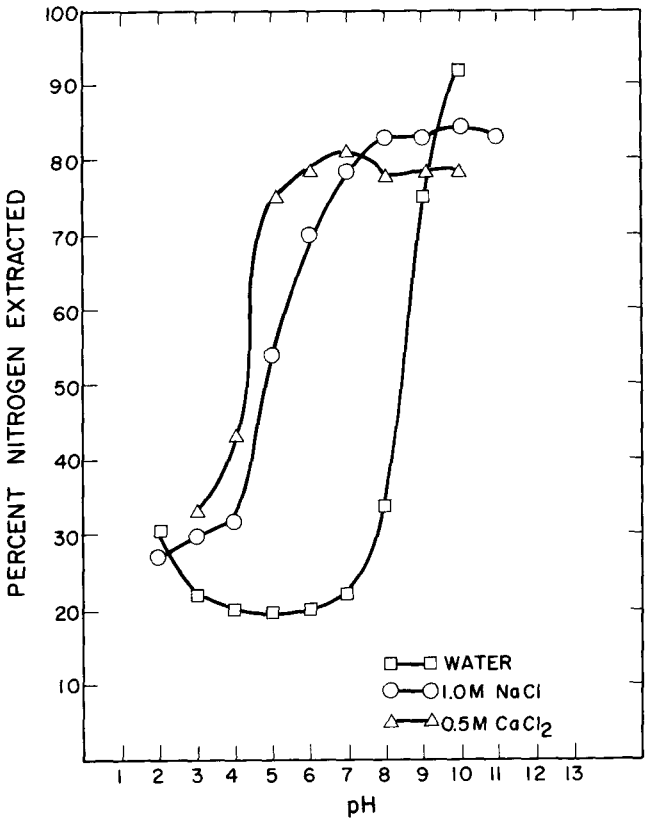

FIG. 3. Effect of 1.0 M NaCl and 0.5 M $CaCl_2$ on the protein solubility profile of sunflower seed meal.

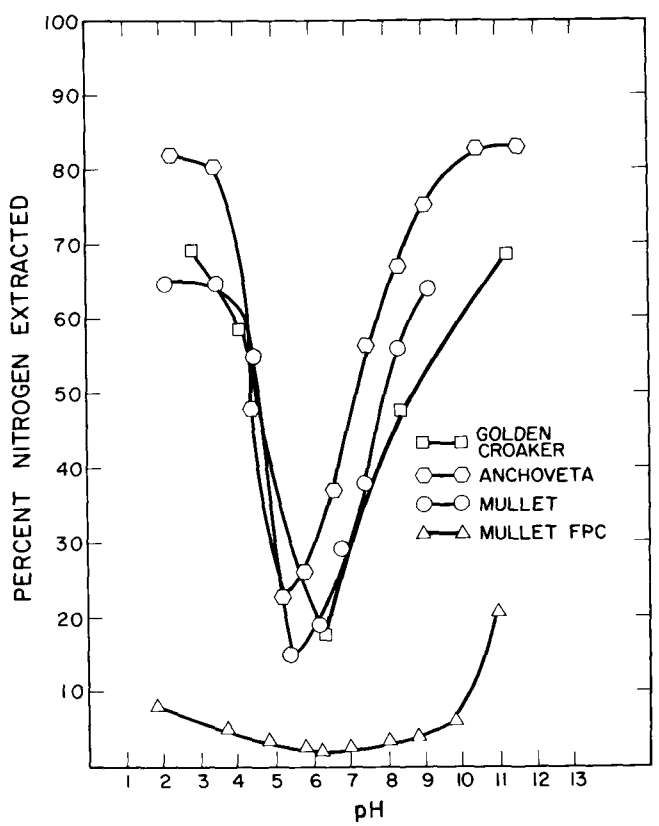

FIG. 2. Protein solubility profiles of several species of fish and of fish protein concentrate prepared from one of the species.

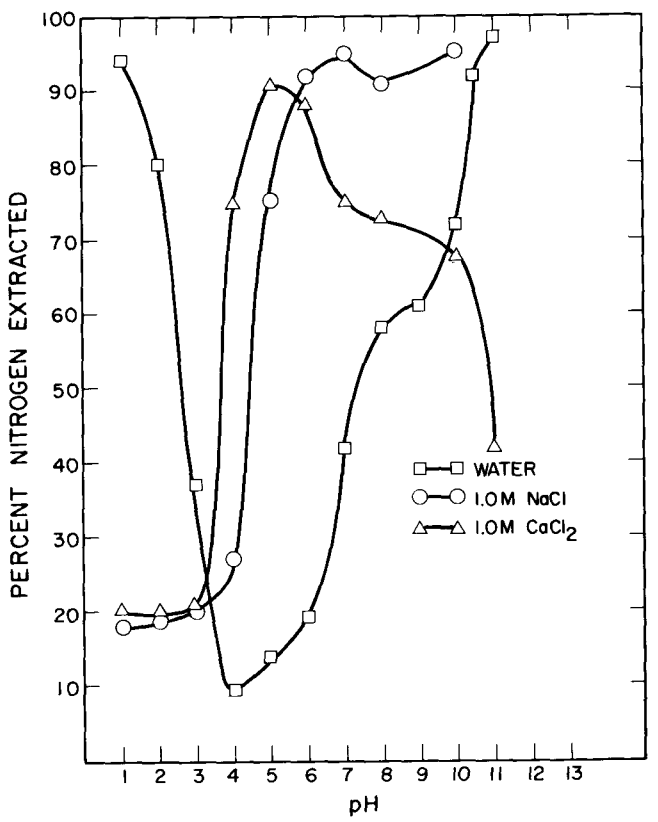

FIG. 4. Effect of 1.0 M NaCl and 1.0 M $CaCl_2$ on the protein solubility profile of coconut meal.

seed meal. What are some of the useful observations that might be drawn from these curves? First of all, it can be seen that the proteins of cottonseed meal and sunflower meal are substantially less soluble at low pH than are those of coconut meal. In this regard, the proteins of coconut meal more closely resemble those of soybean and peanuts. Consequently, it should be possible to produce a protein isolate from coconut meal by extraction at pH 2 and precipitation at near pH 4. All three are quite soluble at alkaline pH indicating that it should be possible to produce isolates by alkaline extraction followed by precipitation at the pH of minimum solubility. It is probably of economic significance that at the point of minimum solubility, more of the nitrogen of sunflower seed meal is in solution than for either the cottonseed meal or the coconut meal. This would anticipate a potentially higher loss in a commercial isolation operation. The inflection in the solubility profile of coconut proteins suggests that two different isolates might be possible, one extractable at pH 8.5 and the other at pH 10.5. In terms of other functionality properties, one might be tempted to deduce that in any food system with a pH of 7 or below, very little of the proteins of cottonseed and sunflower seed would be in solution, and those that are not in solution might be considered to be out of the action. As will be seen later, this could be a mistaken presumption.

In Figure 2 are shown the solubility profiles for several species of fish (W.W. Meinke, private communication). The profiles of fish must be evaluated very carefully unless the history of the raw material is known. Some fish proteins are very sensitive to freezing and if at some point in time the sample has been frozen, a substantial reduction in solubility could have been effected. Our objective has been to obtain the solubility profiles on the proteins of fish in as nearly a fresh state as possible. It is believed that the data in Figure 2 are probably reasonably representative of the fresh state. One would anticipate from these data that any attempt to prepare protein isolates from these fish by traditional procedures would result in substantially lower yields than would be obtained from the oilseed proteins. Nitrogenous constituents of fish are less soluble in both acid and alkaline conditions, and, at the point of minimum solubility, about 20% of the nitrogen would remain in solution. It can be deduced that isolates could be prepared by either acid or alkaline extraction. In preliminary experiments, this has been found to be true and from a practical point of view the acid extraction has some apparent advantages. One can deduce from the solubility profile of the fish protein concentrate (FPC) made by isopropanol extraction of the mullet that the protein has been substantially denatured, assuming that solubility is a measure of some form of denaturation. These data would suggest that in any food system essentially all of the proteins of FPC would be out of solution, and consequently probably inert in terms of most or all of the functionality criteria.

Thus far, reference has been made to aqueous systems containing only the ions naturally present in the protein raw material and added by the NaOH and HCl that were used for pH adjustment. Most food systems contain a variety of ions, either natural or added, and the performance of any protein in such systems could be highly dependent upon the ionic environment. For example, in Figure 3 are shown the effect of 1.0 M NaCl and 0.5 M $CaCl_2$ upon the solubility profile of the proteins of sunflower seed. Note that in this ionic environment, these proteins become highly soluble at pH's near neutral, which are common in many foods. Likewise, in Figure 4 are shown the influence of these two salts on the solubility of the proteins of coconut meal. At acid pH's, these salts suppress the solubility of the coconut proteins. This was also true in the fish proteins that were investigated. NaCl behaves very much the same in coconut meal as it does in

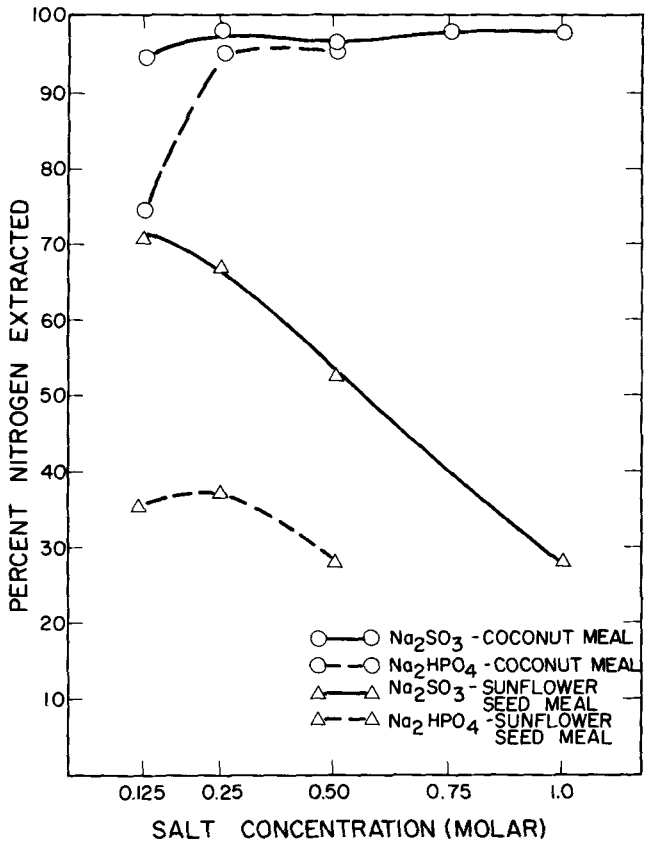

FIG. 5. Effect of various concentrations of Na_2SO_3 and Na_2HPO_4 on the protein solubility profiles of coconut meal and sunflower seed meal.

sunflower meal at the neutral and more alkaline pH's. $CaCl_2$, on the other hand, induced a maximum solubility at pH 5 and a rapidly declining solubility on either side of this pH. These data suggested that it should be possible to produce a protein isolate from coconut meal by extraction with salt solution at neutral pH and precipitation at pH 2. This has been done and the yields were about as would have been anticipated from the solubility profile (5).

This matter of the influence of sodium chloride and calcium chloride on protein solubility is particularly pertinent in foods. Sodium chloride is a common ingredient in many food systems. Further, one of the major potential areas of use of low-cost proteins is in imitation milks. The U.S. Food and Drug Administration has indicated that if imitation milks become an important item of commerce, they will insist that these milks contain a minimum of about 0.03 molar concentration of calcium ions and 0.0055 of magnesium ions. At this time, our research group has no data on the influence of these levels of calcium and magnesium on sunflower seed and coconut proteins. However, Circle (1) has reported that calcium and magnesium ions in this range of concentration greatly suppress the solubility of soy protein. He has reported a protein solubility ranging from 15% to 30% over a pH range from 4 to 9. Fontaine and Burnett (2) have reported similar results for peanut proteins.

These unique solubility interrelationships must certainly be pertinent to any attempt to produce protein beverages, and probably would have a substantial influence on the functionality of proteins in any food systems. For example, if one were to evaluate the whipping property of a protein in an aqueous dispersion, it is uncertain whether the results would predict what this protein would do in the presence of skim milk powder.

At the present time, it is far too early to start making

any generalizations about these solubility interactions. For example, Figure 5 shows the effect of two salts that might commonly be involved in protein food systems on the solubility of the proteins of coconut meal and sunflower seed meal. Note that in increasing concentrations, both salts enhance the solubility of the proteins of coconut meal and suppress the solubility of the proteins of sunflower seed meal.

The points we have raised here certainly compound the problems of those who would evaluate the functional properties of various proteins. The question must always be asked, "In what system; and what is the effect of the other components of the system?" How do the data obtained from an emulsifiability test run in a beaker relate to the complex ionic environment in a frankfurter? Do thickening properties measured by traditional viscosity tests really predict how a protein will act in a meat system? It has been demonstrated that solubility profiles can be used to detect protein denaturation, and to predict modes of preparation of protein concentrates and isolates, but to what extent can they be used to predict functionality in real life systems? We are not aware of any definitive investigations of the effects of other food components, e.g., sugars, starches, lipids, emulsifiers, etc., on the functionality of proteins.

The pioneers in research in oilseed proteins, including Allen Smith, Sidney Circle, Thomas Fontaine and Ray Burnett discovered and disclosed some very significant properties of these proteins. Like many scientific pioneers, they were about a generation ahead of their time. It is possible that many of the modern generation of scientists have not examined the work of these pioneers very closely because, being nearly 30 years old, it might be considered no longer relevant. However, it is suggested that anyone who is truly interested in measuring the functional properties of proteins read the works of these pioneers very carefully. As we review these works and consider our own findings of the past two years, we feel that we are just beginning to know what questions to ask.

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