



## Technical Problems and Opportunities in Using Vegetable Proteins in Dairy Products

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### ABSTRACT

The food processing industry is giving increased emphasis to the production and utilization of alternate protein isolate products as functional and nutritional ingredients in an expanding number of formulated food products. Alternate protein sources such as soy and other vegetable proteins offer additional flexibility in formulating foods due to their economics, availability, functionality and nutritional properties. This paper discusses needs for developing soy and vegetable protein isolates with improved flavor, color and functionality for producing simulated dairy foods. It also considers alternative technologies for incorporating soy and vegetable proteins into the formulation so that they may function properly for forming stable solutions, emulsions, foams and gels that resemble those in their natural dairy food counterparts.

### INTRODUCTION

There are a number of dairy food systems, e.g., fluid milk, infant formula, coffee whitener, sweet and sour cream, margarine, cheese, frozen dessert and whipped topping, which offer opportunities for utilizing vegetable proteins in place of milk protein products (1). Significant progress has been made in developing technologies for utilizing vegetable proteins in a number of simulated dairy food systems; however, several important problem areas must be solved before vegetable protein can be universally used in such dairy food systems. First, it will be necessary to produce "second generation" vegetable protein isolates (2) with improved flavor, color, and functionality compared to those presently available. Second, it will be necessary also to devise new and improved technologies for formulating the simulated dairy food products that will promote the vegetable protein's ability to provide the necessary functional attribute.

Initially, it would be advantageous to gain a thorough understanding of the fundamental properties of the vegetable protein system which control and limit their functionality in each of the application areas. Details on the complexity of the milk protein system, which is the protein system to be simulated by the vegetable proteins, is given by Whitney et al. (3). A similar degree of understanding of the basic properties of the vegetable protein system will be helpful in attempting to overcome technical problems associated with utilizing them in simulated dairy food systems. It is felt that concentrating on the basics offers more promise for solving the serious problem areas than merely attempting to develop a special protein isolate or a special technology for each particular food application.

Since we presently have a more extensive knowledge of the fundamental properties of soy proteins than for any of the other vegetable proteins, this paper will emphasize their technical problems and opportunities for use in simulated dairy food systems.

### THE NEED FOR IMPROVED SOY PROTEIN ISOLATES

Substantial progress has been made in developing functional soy protein isolates with special properties for application in meat systems, coffee whiteners, infant formulas, baked foods and others. Recent reports indicate that soy proteins, consumed at normal dietary levels, compare favorably with animal proteins for meeting human nutritional requirements (4).

As noted before, it will be necessary to produce "second generation" protein isolates which are essentially free of flavor and color components and which retain improved solubility and related functional properties.

Numerous minor organic compounds, present in low concentrations, are responsible for adverse flavor, color, and antinutritional properties of soy and vegetable protein products (5). These inherent compounds, which are co-extracted with the proteins during their isolation, include: carbonyls, phenolics, volatile and nonvolatile fatty acids and their oxidative products, amines, alcohols and various antinutritional factors (2, 5, 6).

A fundamental question remains as to whether the above compounds that are responsible for undesirable flavor in soy protein products are inherent in the intact soybean, or whether they are produced by enzymatic action upon rupture of the cellular structure. Treatment of soy protein products with combinations of heat and organic solvent extraction reportedly improves their flavor (7), but these treatments are not acceptable because they adversely alter solubility and functionality of the resulting product. A practical solution is needed in this area if we are to successfully develop and utilize soy and other vegetable protein isolates in simulated dairy foods.

One approach for preparing soy protein isolates with improved organoleptic properties would be to use the intact soybean as source material rather than defatted flakes, which have previously been subjected to heat and solvent extraction. Another approach for preparing the isolate from the intact soybean would be the "aqueous process" developed for vegetable protein isolation (8).

Since as pointed out by Sosulski (9), phenolic compounds form irreversible complexes with proteins in the pH 4.5-5 range, the acid precipitation step in conventional isolate preparation should be avoided if possible. Further, as shown by Anderson (10), the acid precipitation process results in "acid denaturation" of the proteins, which adversely alters their solubility and functionality. One additional factor which results from acid precipitation of soy protein isolate is the formation of a complex between the protein and phytate (inositol hexaphosphate). This complex causes further deleterious effects on solubility, the tendency to aggregate, and the availability of amino acids for enzymatic modification (11) as well as reducing bio-availability of Zn, Ca and other micronutrients (12). The strong chelating properties of the protein-phytate complex has great significance in the application of soy proteins in simulated dairy food systems if they are to replicate milk proteins with respect to stability to high Ca and Mg ion activities.

In connection with the above indication for the need for improved soy protein isolates, it should be indicated that the economics of processes for producing such improved protein products may not compare favorably with those for conventional processes. However, the essentiality of this need for improved protein products dictates that such processes be developed and implemented if we are to successfully utilize soy and vegetable proteins in simulated dairy food systems.

### PROPERTIES OF MILK PROTEINS

The major proteins in milk, the caseins and whey proteins, have been extensively studied with respect to their nomenclature, conformation and chemical properties (3). Also, the possible relationships between these basic properties and their functional properties in food systems has been proposed (13). It is therefore appropriate to review briefly the properties of the milk proteins in order to indicate differences and similarities with soy and vegetable proteins and to suggest plausible approaches for improving soy and vegetable proteins for use in simulated dairy food systems.

The caseins, which comprise about 80% of the total milk proteins, exist in milk and natural dairy products as structured complexes which contain substantial amounts of colloidal calcium phosphate, termed micelles. The micelle system has been established as the essential component of milk for stabilizing the high levels of colloidal calcium phosphate during the processing and handling of dairy food products. An understanding of the chemistry of the casein micelle system is essential for explaining the fundamental properties of milk proteins and their role in the formulation and processing of milk and milk products (14). In addition, commercial caseinates, which are isolated from milk by a process similar to that used for isolating soy proteins, contain no micellar structure and thus do not perform all of the functions attributed to casein micelles in milk.

Each of the three major caseins, e.g.,  $\alpha_s$ -,  $\beta$ -, and  $\kappa$ -casein, contain different numbers of ester phosphate and carboxyl groups per monomer subunit (3) which largely accounts for their unique chemical and functional properties. For example,  $\kappa$ -casein, which contains only one ester phosphate group, one disulfide group, and up to five carbohydrate chains per monomer subunit, exhibits highest stability to Ca ions, and in fact serves as the stabilizing factor for the entire casein micelle structure in milk. Conversely,  $\alpha_s$ -casein, which contains eight ester phosphate groups per monomer subunit, and contains no disulfide or carbohydrate groups, exhibits highest Ca sensitivity.

Since the primary structures have been established for each of the major casein subunits, it is possible to develop conformational models to explain their excellent functionality in a wide range of formulated food systems, especially those that comprise emulsion and foam systems. Casein subunits have been termed "amphiphilic" molecules due to the pronounced segregation of the hydrophobic and hydrophilic regions along their polypeptide chains (15). It has been proposed that their ability to undergo rapid association-dissociation in food systems may be due to the specific orientation of their monomeric subunits within the complexes to retain a high degree of availability of their carboxyl and ester phosphate groups for reaction with the aqueous medium (13). These complexes rapidly dissociate to provide monomer units that stabilize freshly formed oil-water and air-water interfaces during homogenization or whipping (16). The ability of the complexes to undergo rapid dissociation is strongly influenced by pH and other ionic environment conditions, thus these compositional factors have a significant effect upon the functional properties of the caseins.

Since caseins are relatively free of disulfide groups, e.g.,

$\kappa$ -casein is the only component that contains a disulfide group, and since they contain little if any secondary or tertiary structure, it is not surprising that they are relatively insensitive to heat and other processes which promote protein denaturation and aggregation. Thus, the caseins retain most of their functionality, even in food systems subjected to extensive heat processing.

Whey proteins, however, contain substantial numbers of sulfhydryl groups (3) and also possess secondary and tertiary structure. Thus, upon denaturation they normally undergo aggregation which reduces their solubility and related functionality (17). Denatured whey proteins, mainly  $\beta$ -lactoglobulin, also interact with  $\kappa$ -casein, concentrated mainly within the casein micelle structure in milk, or within caseinate complexes in simulated food systems, via formation of intermolecular disulfide bonds. These interactions between the whey proteins and casein micelles play an important role in providing heat stability to milk proteins in highly heated milk products (14).

### PROPERTIES OF SOY PROTEINS

Although the nomenclature and related chemical and functional properties have been established for milk proteins, such information has not been sufficiently developed for the extremely complex soy and vegetable protein systems. Since most experimental and developmental work has been with crude or partially purified and characterized soy and vegetable protein preparations, it is difficult to develop models and mechanisms for explaining variations in chemical and functional properties of these proteins in food systems.

The soy globulins, which have been extensively studied, are commonly designated: 2S (15%), 7S (34%), 11S (42%), and 15S (9%) on the basis of sedimentation in centrifugation experiments (2). On the basis of additional basic research findings, it has been established that each of the major soy protein components, e.g., mainly the 7S and 11S proteins, contain a number of subunits which undergo association-dissociation reactions in solution and thus contribute a significant amount of quaternary structure and complexity to the system. Interactions among these subunits probably have significant effects upon the functional properties of soy proteins in food applications. The soy proteins are also highly susceptible to processing treatments that involve heat, drying, and exposure to polar organic solvents. This latter sensitivity to denaturants is undoubtedly related to their significant levels of sulfhydryl and disulfide groups as well as their secondary and tertiary structure. They resemble milk whey proteins in their susceptibility to denaturation and subsequent aggregation reactions, which largely involve rupture and reformation of intermolecular disulfide and hydrophobic bonds. These latter reactions result in a substantial loss of solubility and functionality of soy and vegetable proteins in food applications. It is therefore important to produce soy protein isolates with a minimum of denaturation, especially for those applications that require a high degree of solubility and related functionality.

Also, as indicated above, it would be advantageous for the soy and vegetable isolates to function in most cases as the caseins do in milk and natural dairy food systems. Thus, although denaturation of soy proteins during their isolation and production undoubtedly inhibits subsequent solubility related functionality, it is likely that "in process" denaturation, produced at the appropriate stage in the formulation process, can be used to improve certain functional properties of soy proteins, as has been accomplished with milk whey proteins (18).

It will probably be necessary to further establish the nomenclature of the soy proteins in order to explain mechanisms for their loss of functionality by heating in

certain food applications. Although amino acid sequences have not been determined for the major soy protein components, it may be assumed from their similarity to milk whey proteins with respect to chemical and functional properties, that they possess a random amino acid distribution along their polypeptide chains. Such a uniform amino acid distribution pattern may favor the formation of heat-induced protein complexes with reduced solubility and functionality due to the occlusion of significant proportions of polar groups inside the complex. Although data is lacking in this regard, the possible random distribution of hydrophobic and hydrophilic groups along the soy protein subunit's polypeptide chains might also explain their relatively poor capacity to stabilize emulsions and foams, since this conformation would presumably not provide sufficient surfactant properties to permit them to be strongly adsorbed at the interfacial regions of the disperse phases.

Appropriate modification treatments, such as limited enzyme hydrolysis to reduce molecular weight, alter hydrophobicity, or to introduce ester phosphate groups into the molecules at random or specific sites along the polypeptide chains might alter the soy proteins sufficiently to impart improved functionality characteristics. As more information becomes available on the nomenclature and basic chemical properties of soy and vegetable proteins, it may be possible to control such modifications to produce protein products with improved functionality for simulated dairy food systems.

#### PRODUCTION OF SIMULATED DAIRY FOOD SYSTEMS

As indicated above, the successful production of simulated dairy food products will require soy or vegetable protein isolates with significantly improved flavor, color, and functional properties. The need for such high quality protein products stems from the fact that milk and most dairy food products have an extremely mild and delicate flavor, low color level, and exhibit a characteristic texture that will not accommodate even a minor alteration. Even though certain fermented dairy foods and cheese products possess a highly viscous or gelled structure and have a characteristic flavor and color, soy and vegetable protein products must not detectably alter these properties or they will be readily identified as inferior products by the consumer.

Processing technology innovations will also be needed to successfully produce simulated dairy food products using soy and vegetable proteins. Properly established procedures for solubilizing, blending and incorporating soy and vegetable proteins into the formulation, which will presumably replicate the composition and overall properties of milk or the other natural dairy food system, will be essential for successfully utilizing these proteins in simulated dairy food

systems. Since soy proteins are quite sensitive to pH and ionic activity levels, it will also be necessary to control correctly these factors to provide the protein molecules and complexes with the correct charge and related conformational properties, thereby enabling them to provide the necessary functionality. In this latter regard, it might be useful in certain applications to provide those conditions during the formulation process that would result in the formation of soy protein-calcium phosphate complexes which would resemble casein micelles and complexes in milk and other natural milk products. Such complexes could presumably be converted into cheese curd by treatment with coagulating enzymes much as casein micelles are reacted in milk. These simulated cheese products might resemble the natural cheese counterpart more closely than if produced from an aqueous dispersion of the soy protein isolate. If properly assembled, such soy protein-calcium phosphate complexes should exhibit sufficient heat stability to permit their use in simulated sterile milk products and infant formulas. It would probably be important if these soy protein-calcium phosphate complexes would be capable of undergoing rapid association-dissociation reactions such that they could release sufficient protein monomer units to adsorb at oil-water and air-water interfaces and thereby serve as a significant stabilizing factor for emulsions and foams, much as the caseins are able to produce.

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