Methods of Studying Functional Characteristics of Vegetable Proteins

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ABSTRACT

In a functional characterization, a set of data is obtained which gives information on the fields of application for a certain protein ingredient. This fingerprint can be used as a guideline in product development and restricts the amount of tests on large scale. The function of a protein in a complex food system can be better understood if a functional characterization is combined with a microstructural investigation. This may be especially important in cases where proteins act by indirect functionality, i.e., when the prescence of one protein changes the function of another component. In order to improve the efficiency of functional characterizations, methodologies need to be further developed. This is especially important for characterization of gels, emulsions, and mixtures of structures.

INTRODUCTION

Among the most important aspects of vegetable proteins are their functional properties. These will determine the field of application as well as whether a new protein ingredient will be competitive on the market.

Before discussing the problems and possibilities of functional characterization, a definition is appropriate: "functional properties are physico-chemical properties which give information on how a protein will behave in a food system." Properties such as solubility, swelling, viscosity, texture, water- and fat-binding, emulsion, foam, and gel characteristics are of general interest. As functional properties are determined in order to predict the behavior of protein ingredients, we need accurate knowledge of methods and experimental conditions. Proteins take part in very complex food structures, and it is important to know the specific function of a protein ingredient. Extensive research is also needed to make a proper characterization of food structures.

THE TRIAL-AND-ERROR TECHNIQUE

If specific functional properties cannot be determined, one has to rely solely on the so called trial-and-error technique. This is the most traditional way to test a new ingredient, and it means that the ingredient is tested directly in various types of foods. This approach is time-consuming and will, in the long run, turn out to be expensive and inefficient. The drawbacks of the trial-and-error technique can be summarized as follows: (a) requires large quantities of raw material; (b) limited information is obtained about the function of the ingredient being investigated; (c) minor changes in processing conditions or the recipes may have unexpectedly large effects the final food properties; (d) it is difficult to control the fat and water content of the final product without proper knowledge of the function of the ingredients.

Let us look at some examples.

Suppose that two ingredients are to be compared in a sausage formula of frankfurter type. If the trial-and-error technique is used, the unknown ingredients are tested directly in an industrial recipe. This probably means a rather low concentration of the ingredients and the presence of several other ingredients. The meat used is a mixture, and the quality variations may be considerable. A batch is at least 30 kg or may be as much as 500 kg.

If a difference between sausages containing the two ingredients is noted, it is very difficult to say whether it is due to different functionality of the protein ingredients; minor variations in processing conditions, e.g., temperature; variations in the meat quality; variations due to other ingredients. Consequently, a large number of tests have to be made in order to establish a difference between the two ingredients in the sausage formula.

This type of test gives no information on the specific function of a protein, and lack of that knowledge makes it difficult to handle the protein in the right way. If a protein is used because of its gelling properties, the gelation temperature must be known. Otherwise the product may not be heated to the critical temperature. The protein will then give no contribution, and it may even have a negative effect on the final food properties.

A protein may also contribute to the structure and water-binding properties with high viscosity which will paste meat particles together. If the viscosity is highly concentration dependent, the meat quality will influence the function of the protein. If the meat has poor water-binding properties and much water is released from the meat during cooking, the protein may lose its pasting ability due to the decrease in concentration.

FUNCTIONAL CHARACTERIZATION

In order to obtain the information necessary for the choice and handling of protein ingredients, systematic studies have to be made with simple model systems. For a proper functional characterization, combinations of several data are necessary. The experimental design is instrumental in giving a fingerprint of the protein. The pattern of data rather than individual measurements gives information on functionality. With a model study, the function of a protein ingredient is more easily understood and the number of large scale tests can be restricted. It is important to find out at an early stage the properties that are relevant for a certain application. Functional parameters can then be used in process optimization.

Status of the Protein

Before starting a functional characterization, it may be of interest to have some information on the status of the protein. Properties of proteins are not only determined by their origin, but also be processing conditions during production as well as by the presence of other components as illustrated in Figure 1. Sometimes the processing conditions play a dominant role for the functional properties. It is then not possible to make general statements on the functionality of a certain type of protein. The degree of denaturation caused by processing has an impact on most functional properties. A good way to determine the degree of denaturation is by means of differential scanning calorimetry (DSC). In this method the temperature is increased linearly and the energy required for protein denaturation is measured vs. a reference sample. Denaturation is observed

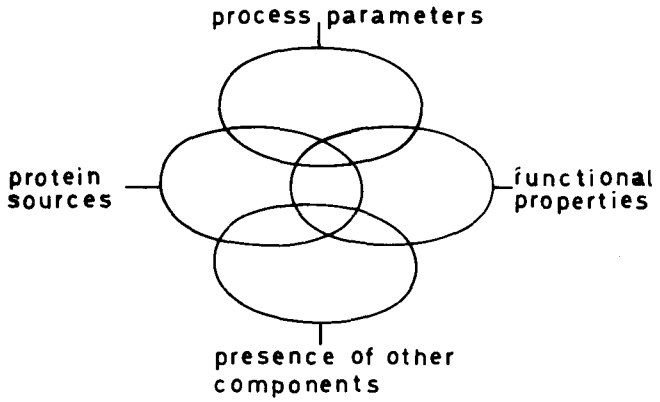

FIG. 1. A Venn-diagram illustrating the complex dependence of functional properties on various factors.

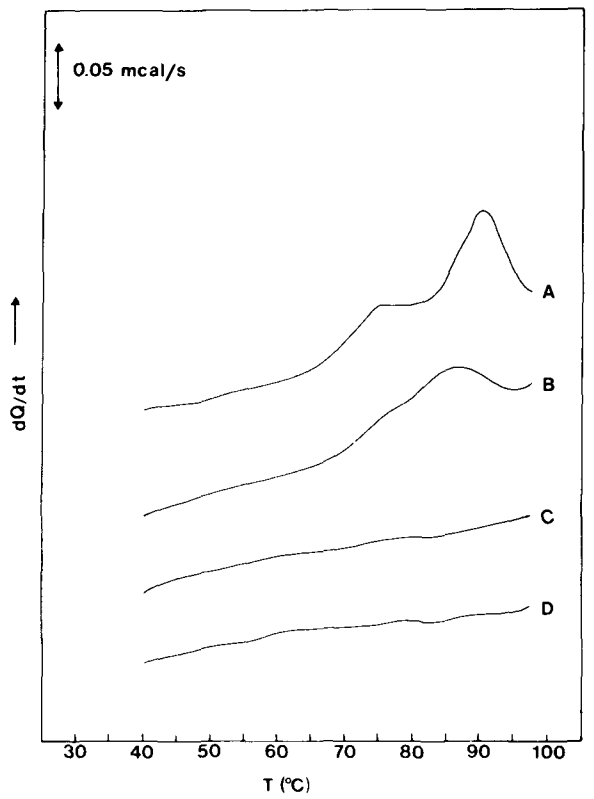

FIG. 2. DSC thermograms of 10% soy protein isolates at pH 7.0 in distilled water. Isolate A - produced under mild conditions on pilot plant scale. Isolates B, C, and D - commercially available isolates. The sensitivity was 0.5 mcal/s and the heating rate 10 C/min.

as an endothermic peak, and the area under the peak corresponds to the enthalpy change involved in the reaction. If the protein is already partly denatured, the area under the peak will decrease, and if it is completely denatured no peak will appear.

Figure 2 shows DSC thermograms of four soy protein isolates. Isolate A is produced on pilot plant scale under mild conditions. Isolate B, C, and D are commercially available isolates. The thermogram of isolate A shows two peaks whereof the first corresponds to the 75 and the second to the 115-globulin (1). Isolate B has been partly denatured during processing. The two peaks cannot be clearly distinguished, and the total area is less than that of isolate A. Isolates C and D are both completely denatured. This does not mean a lack of functional properties. On the contrary, both isolates C and D possess good water-binding properties.

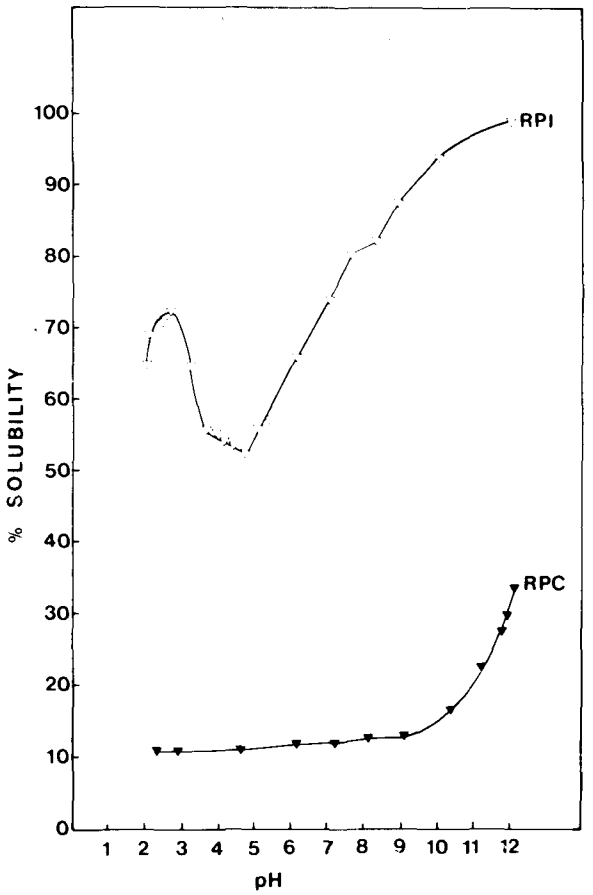

FIG. 3. Solubility as a function of pH of 1% dispersions of a rapeseed protein isolate (RPI) and a rapeseed protein concentrate (RPC) in 0.2 M NaCl.

Solubility

Most functional properties are determined by the balance between forces underlying protein-protein and protein-solvent interactions. This balance is affected by changes in pH, concentrations, temperature, nature of solvents, presence of other components, etc.

Most of these parameters have an influence on the solubility of the proteins. Solubility has therefore also been used as a measure for other properties. However, even if a change of two properties is caused by the same underlying mechanisms, it does not mean that the properties themselves are correlated.

Colloidal stability is often used as a measure of protein solubility. Relative methods are used, where the amount of nitrogen in the supernatant is measured after centrifugation. Experimental conditions such as mixing procedures, centrifugal force and protein concentration should be kept under control (2,3). If foaming occurs care should be taken as proteins are enriched in the foam phase.

Solubility measurements give valuable information on a protein ingredient. The pH-dependency reflects the complexity of a protein system if several maxima, minima, and inflection points are found. Figure 3 shows the solubility profile of a complex rapeseed protein isolate produced by gelfiltration. In Figure 3 the solubility of a rapeseed protein concentrate produced from the same raw material is also shown. The effect of processing is obvious. Such a solubility profile with a low solubility over a broad pH-range is a sign of severe denaturation and insolubilization.

Denaturation and solubility are not always correlated. High solubility data are sometimes obtained from completely denatured proteins. Figure 4 shows the solubility of soy protein isolates A and D. The corresponding DSC

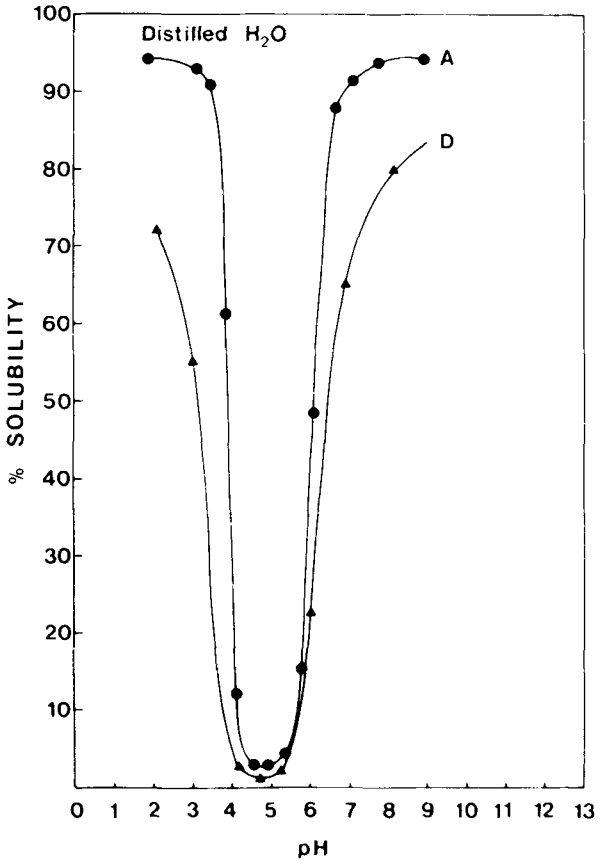

FIG. 4. Solubility as a function of pH for 1% dispersions of the mildly produced soy protein isolate A and the commercial soy protein isolate D in dist. water.

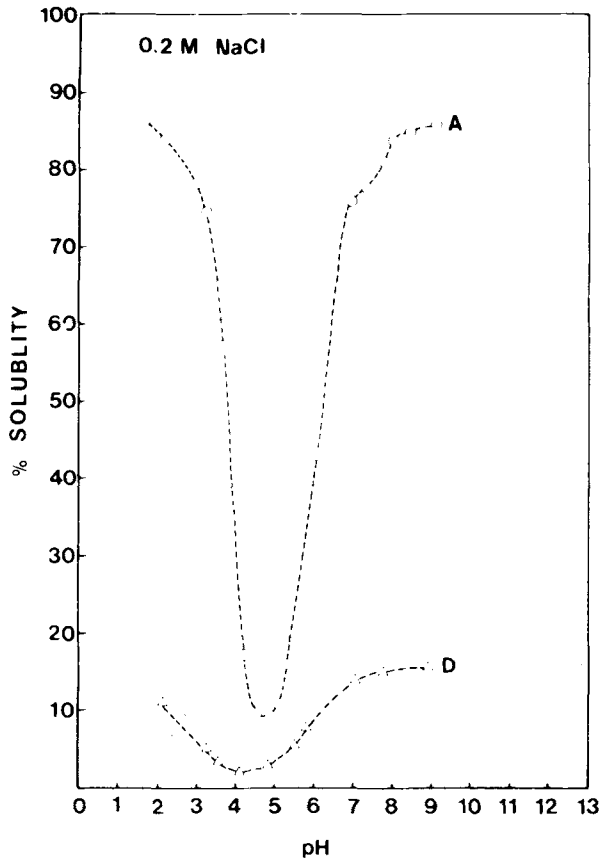

FIG. 5. Solubility as a function of pH of 1% dispersions of the mildly produced soy protein isolate A and the commercial soy protein isolate D in 0.2 M NaCl. (2).

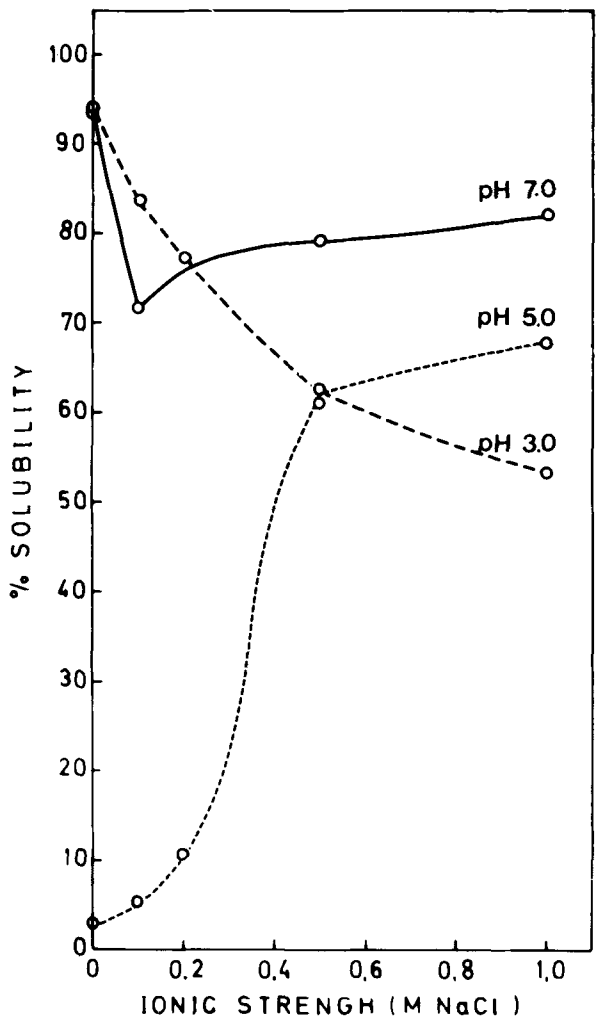

FIG. 6. Solubility as a function of NaCl concentration at pH 3.0 5.0, and 7.0 of 1% dispersions of the mildly produced soy protein isolate A. (2).

thermograms are shown in Figure 2. The denatured isolate D shows a solubility profile closely related to that of the native soy protein isolate A.

These measurements were made in distilled water. The presence of 0.2 M NaCl results in a marked decrease in the colloidal stability as seen in Figure 5.

In functional characterization the effects of salt have to be considered. The salt effect is pH dependent (2,4). Figure 6 shows the solubility as a function of NaCl concentration at pH 3, 4.5 and 7 for the native soy protein isolate A.

Sodium chloride can be used to get a general idea of the salt sensitivity. However, ions may have highly specific effects. If a protein is to be used in a beverage, small concentrations of calcium may cause problems. The reason is seen from Figure 7, which shows the solubility of isolate A as a function of pH at different $CaCl_2$ concentrations. The effect of small concentrations of $CaCl_2$ on the solubility is pronounced on the alkaline side of the isoelectric region (1,5).

Swelling and Viscosity

Solubility measurements give no information whether a protein ingredient will bind water or contribute to the texture of a product. Water-binding may be caused by any of the following properties: (a) the ability to swell and take up water; (b) a high viscosity caused by soluble molecules, swelled particles or a mixture; (c) the ability to form a gel network during processing.

Swelling, when defined as the spontaneous uptake of water, is the first step in the solvation process. If swelling is

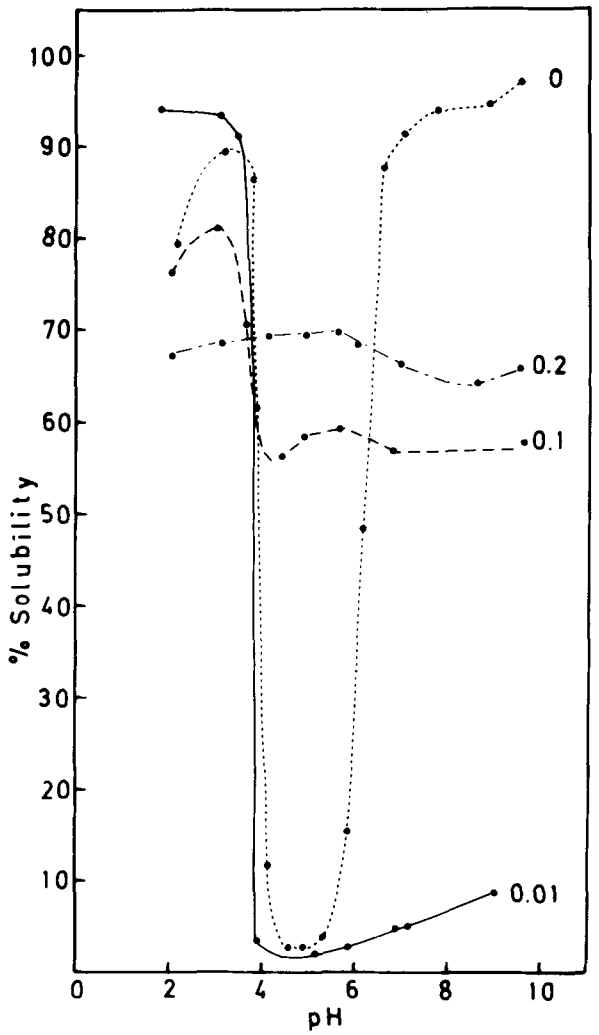

FIG. 7. Solubility of 1% dispersions of the mildly produced soy protein isolate A as a function of pH at 0, 0.01, 0.1, and 0.2 M $CaCl_2$ (1).

unlimited, the protein will solvate, otherwise swelling will proceed until it is limited by various intermolecular forces in the swollen sample. Figure 8 illustrates the difference between the unlimited and the limited type of swelling. A sodium caseinate shows an unlimited type of swelling, where the particle structure is quickly destroyed. A soy protein isolate shows a limited type of swelling, where the particle structure is resistant to several hours of swelling (6). Only empirical methods are available for the measurement of swelling. One way is to measure the spontaneous uptake of water in a glass capillary as shown in Figure 9.

The glass equipment consists of a thermostated funnel connected to a horizontally located capillary. A sample is dusted on to a wetted filter paper which is fastened on to a glass filter placed on top of the funnel which is filled with liquid. The uptake of fluid can then be followed in the capillary (6).

Various types of instruments are available for viscosity measurements such as capillary, coaxial cylinder and plate and cone viscometers (7,8). Most studies on protein dispersions have been made in coaxial cylinder instruments, where one of two cylinders is rotated, and the torque from the sample in the gap is registered. Provided the geometrics are well defined, and the gap small, the torque and rpm are proportional to shear stress and shear rate. By measuring shear stress vs. shear rate a flow curve is obtained which enables calculations of the flow characteristics.

Most protein dispersions at high concentrations are non-Newtonian, which means that there is no linear relationship between shear force and shear rate. Flow curves of a soy protein dispersion at various concentrations are shown in Figure 10.

Therefore, measurements have to be made at several shear rates. In the newer viscometers, flow curves can be registered directly on an XY-recorder as the shear rate can be continuously increased during a measurement. A common problem in flow characterization of protein dispersions is their time dependency. It is especially complicated if the time dependency is irreversible. The results will then depend both on the prehistory of the sample and of the actual design of the measurements. Even if control of experimental conditions is essential, there is a fair amount

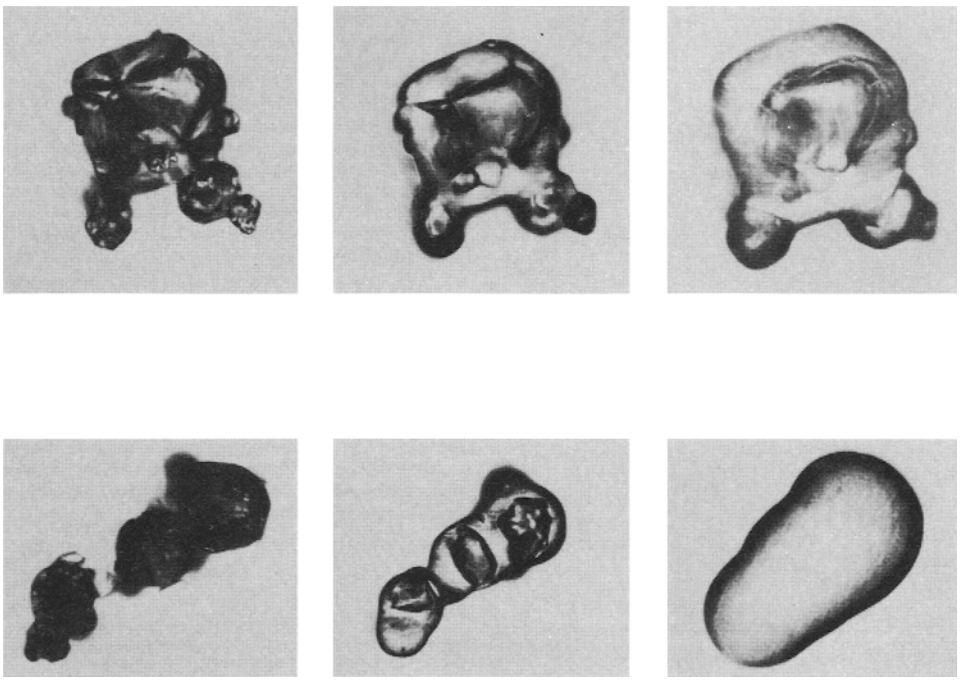

FIG. 8a. Micrograph of soy protein isolate B after 0, 1 resp. 5 hr of swelling, (6). FIG. 8b. Micrographs of sodium caseinate after 0, 5 resp. 30 min of swelling (6).

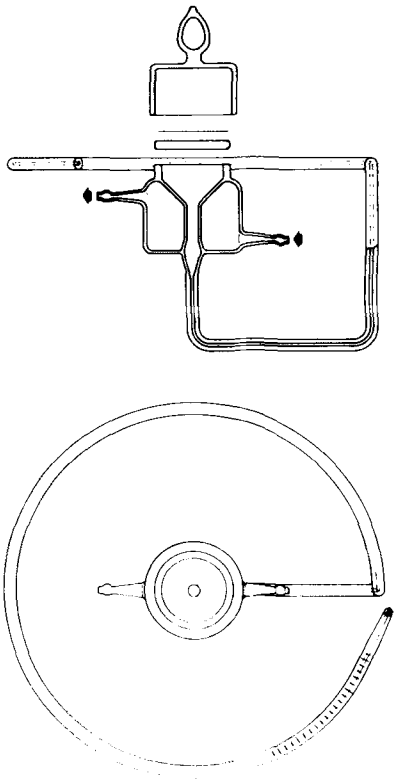

FIG. 9. Equipment for measuring the spontaneous uptake of water.

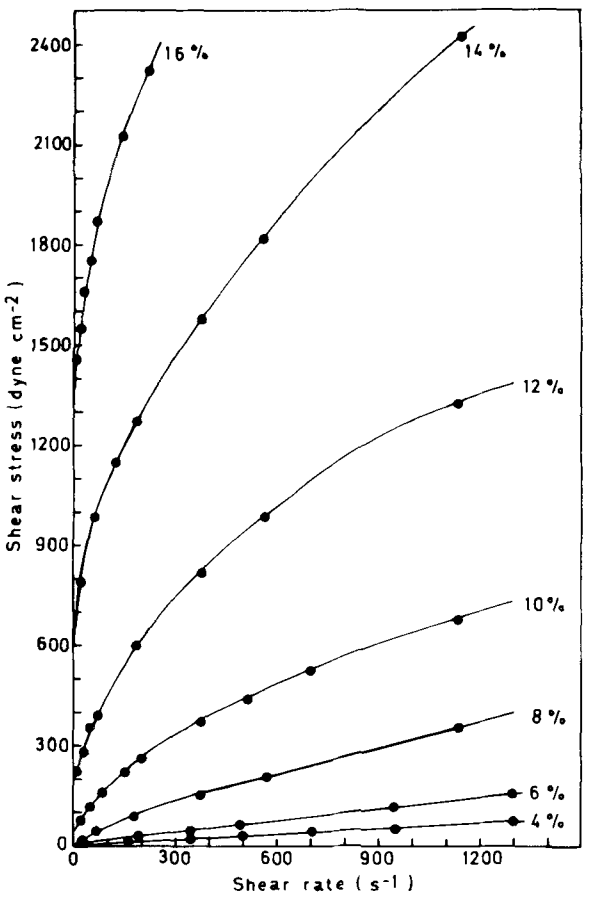

FIG. 10. Flow curves at various concentrations of soy protein isolate B in dist. water. (9).

of knowledge on how to use solubility, swelling, and viscosity data in a functional characterization. It is always important to consider pH, ionic strength and other variables relevant for a certain application.

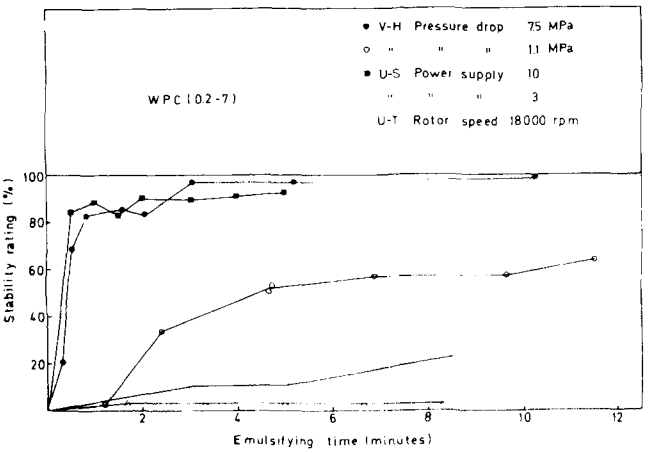

FIG. 11. Stability of whey protein emulsions as a function of the emulsifying time when emulsified with different equipment at various intensities. V-H = value homogenizer; U-S ultra sonic, U-T = Ultra turvax (10).

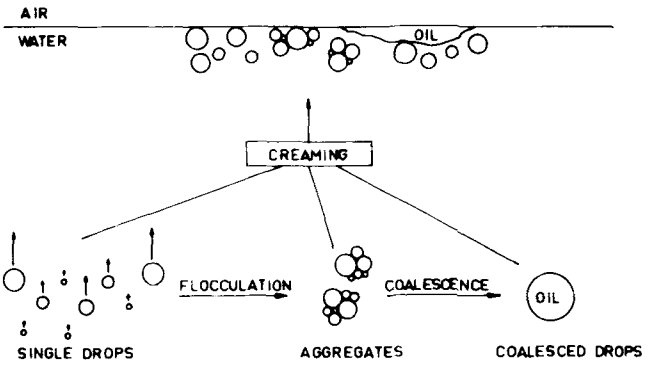

FIG. 12. A schematic representation of emulsion instability (11).

STRUCTURE FORMATION

There is less knowledge on the functions of proteins during structure formation. The aim of most food processors is to create some kind of structure which gives the food item its special characteristics. There are numerous examples from the boiling of eggs to more complex food processes such as the production of sausage, cheese, bread, cakes, mayonnaise, and whipped products. The structures or states of aggregation formed during processing can be divided into gels, emulsions, foams, and suspensions.

Many food products are made out of complex combinations of these structures. In a sausage the protein gel network is the main structure. If part of the fat is liquid, it can be emulsified during chopping, and proteins then act as emulsifiers. It is well known that the presence of solid particles may have a stablizing effect on the final structure. If air is incorporated during chopping, a foam may also contribute to the complex structure of a sausage.

One of the most important aspects of functional characterization should be to find out how a protein will take part in and influence the formation of structures. However, there is still a lack of knowledge in this field. The very important emulsion and gel structures are far from evaluated, and a good description of the protein function is difficult to make.

Emulsions

A reason for the difficulties in evaluating the emulsifying properties of proteins is that the process of making

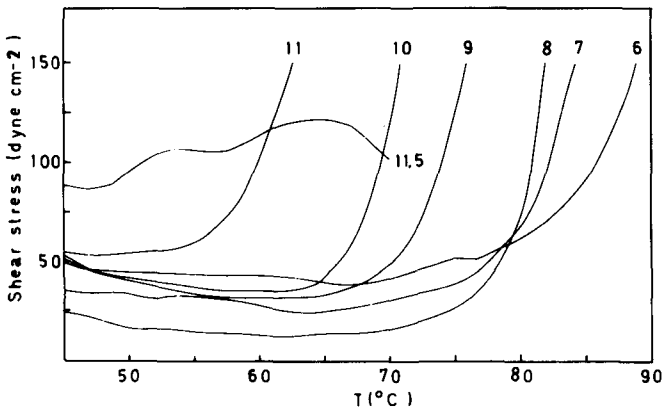

FIG. 13. Shear force at 300 s^{-1} as a function of temperature of 10% soy protein A dispersion in dist. water at pH 6.0 - 11.5 (1).

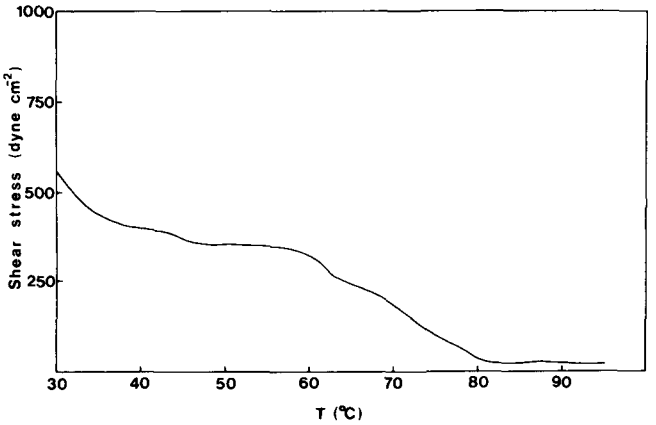

FIG. 14. Shear force at 300 s^{-1} as a function of temperature of a 10% soy protein D dispersion in dist. water at pH 7.0. (16).

the emulsion often plays a dominant role. Figure 11 shows the stability against creaming as a function of various processing conditions of emulsions with exactly the same composition.

When oil is split in a water phase to form an emulsion, the splitting mechanism is mainly determined by the process. The proteins stabilize the emulsion by diffusing to the interface where they form an interfacial membrane. The particle size of the oil droplets will depend on the spliting mechanism caused by the process and the ability of the protein membrane to prevent coalescence of oil droplets.

Emulsifying capacity and emulsion stability measurements are often used in functional characterizations of protein. There is a big difference between these two terms. Emulsifying capacity denotes the maximum amount of oil that can be emulsified by a protein dispersion, whereas emulsion stability denotes the ability of an emulsion with a certain composition to remain unchanged. Stability of an emulsion can appear visually as creaming and fat separation. The underlying mechanisms of instability are flocculation and coalescence as illustrated in Figure 12.

As the emulsification process plays a dominant role with respect to the emulsion properties, it is difficult at this stage to define the function of specific proteins from simple emulsion characteristics. However, basic research has been made on the interfacial behavior of proteins in relation to the emulsification process. This work includes dynamic surface tension measurements, determinations of particle size distributions and the amount of protein adsorbed per unit area of fat (12-15).

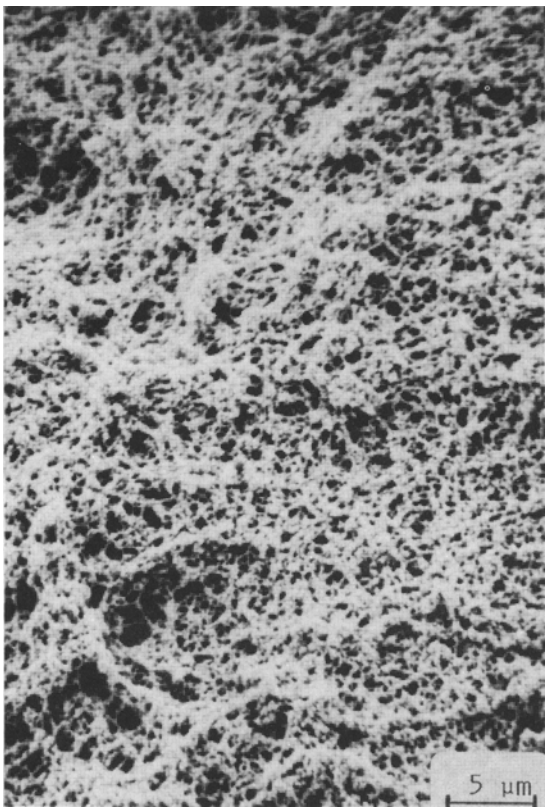

FIG. 15. Scanning electron micrograph of a 10% whey protein gel in dist. water. Chemical fixation was made with glutaraldehyde following by critical point drying. (16).

Gels

The most important structure in semisolid and solid food products is the gel or three-dimensional network structure. The network structure determines many rheological parameters and acts as a matrix holding not only water but also lipids, dissolved proteins, and carbohydrates as well as other species. The most common food gels are induced by heat treatment. Gel formation can also form spontaneously, e.g., by swelling at high protein concentrations. Knowledge is required on gelation mechanisms, gel structures, and gel properties such as water-binding and texture. Mechanisms involved in gel formation are determined by the balance underlying protein-protein and protein solvent interactions. Several reactions may be involved in gel formation such as swelling, solubilization, dissociation, association, and aggregation (1). Depending on the conditions and the type of protein, the relative importance of the reactions may vary and thereby give rise to different types of gel structures.

In the case of native soy proteins, the denaturation and dissociation steps prior to aggregation play an important role for the nature of the gel network (1). However, firm gels can also form from commercial soy protein isolates, which are completely denatured according to DSC measurements. Not only gelation mechanisms but also the resulting gel structures and gel properties will differ between these two types of soy protein gels. Figures 13 and 14 give an example of the difference in behavior. When the viscosity was measured as a function of heating temperature, a viscosity increase was observed for undenatured or partly denatured proteins at temperatures correlated with the denaturation temperatures (1,16). When a commercially available denatured soy protein isolate was tested under exactly the same conditions, the viscosity decreased rather than increased as can be seen in Figure 14. In spite of the viscosity decrease, the commercial isolate formed firm gels after cooling.

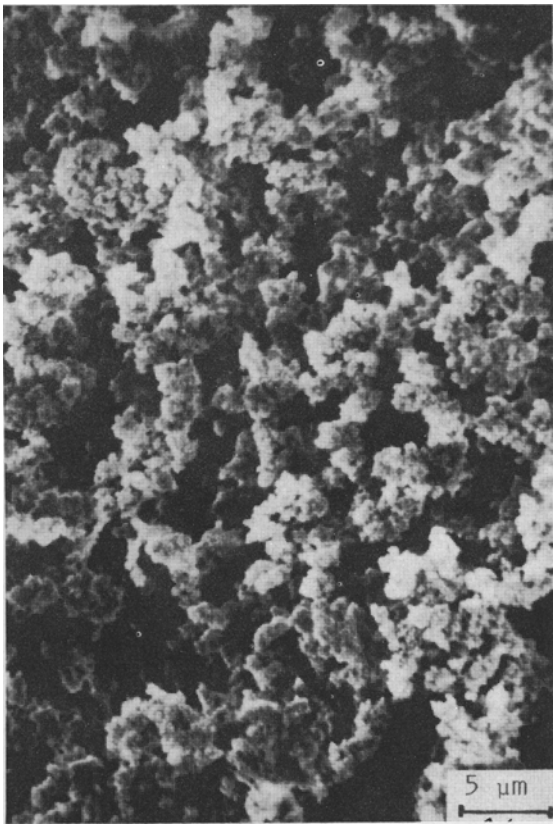

FIG. 16. Scanning electron micrograph of a 10% whey protein gel in dist. water. Chemical fixation was made with glutaraldehyde followed by critical point drying. (26).

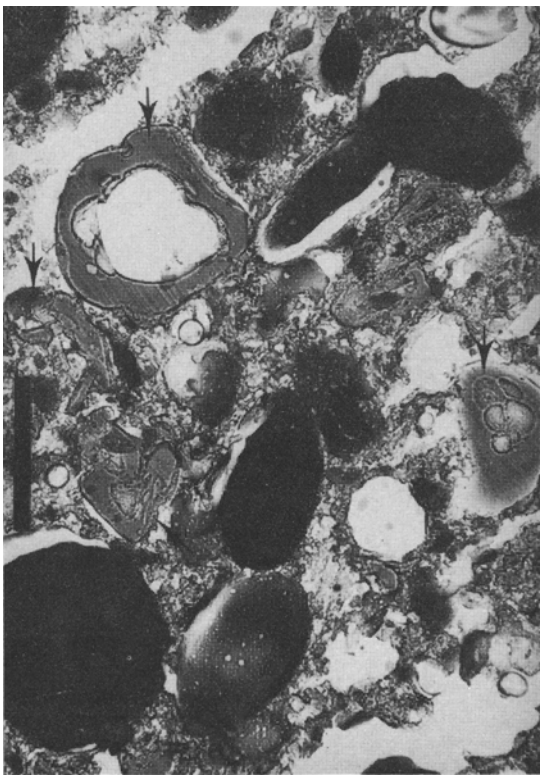

FIG. 17. Light micrograph of a meat batter with 6% soy protein isolate (D). The arrows show the prescence of swelled soy protein particles. The length of the line cooresponds to 100 µm. (23).

The most important gel properties are water-binding and texture. Far too little has been done on the methodology of these properties with regard to gels. Most water-binding studies have been made on meat systems. Generally the water release is measured as a function of an external force such as pressure or centrifugation (17). An interesting alternative approach is to measure water-binding by capillary suction (18,19). The method developed by Labuza and Lewicki (19) has been tested on some gel systems. The water-binding is measured as the withdrawal of water by a capillary standard material (filter papers) in contact with the gel. The method has not yet been tested on gels from globular proteins.

Relative measurements on gel texture have been made by penetration and compression tests (20, 21, 22, 23). Defined viscoelastic measurements have been made on a few protein systems (24, 25). No publications on systematic studies have been made to find out which gel texture characteristics are important for the function of proteins in complex food systems. Few measurements have been made on gels, and there is a common misunderstanding that gel firmness and the water-binding of gels are correlated. This is not always the case. Soft gels with a fine network structure may have better water-binding properties than firm gels with a coarser network structure.

Structure-Functional Relationships

The type of structure that is formed determines many of the functional properties. By a functional characterization, we know that a protein possesses certain properties, but it is more complicated to find out why. When the reason is known, the functionality can be understood and alterations are easier to make. One way of characterizing the structure is by microscopy. The gel structure can be revealed by electron microscopic methods. It is known that gel properties such as texture and water-binding are strongly affected by the presence of salts. Figures 15 and 16 show electron micrographs of gel structures of whey proteins formed under exactly the same conditions with the exception that the structure in Figure 15 was formed in the absence, and the structure in Figure 16 in the presence of 0.2 M NaCl.

It is now easy to understand why the gel made in distilled water has better water-binding properties than the coarser gel made in 0.2 M NaCl (26). It is not always necessary to use expensive electron microscopes in order to visualize the specific function of a protein. In Figure 8 it was demonstrated that soy protein isolates have a function due to their swelling properties. As the swelling was regarded as limited, one would also expect to see the presence of swelled particles in a meat system. Figure 17 shows a light micrograph of a meat batter containing a soy protein isolate. The presence of swelled particles is easily identified.

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