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# Using protein separation techniques to understand protein performance in food systems

An understanding of the performance of protein in a food system is crucial to both quality control and new product development. Proteins are an important component of most foodstuffs, and, along with fats and carbohydrates, constitute the three macronutrients. The functional properties of proteins can be affected by their chemical structure, processing treatments (chemical and enzymatic modification of molecular weight and solubility) and the environment in which they are applied (concentration, pH, temperature, mixing method and duration).

A wide range of techniques are used to study the structure, functionality, and impact of processing on proteins in food products. These include polyacrylamide gel electrophoresis (PAGE) and size exclusion chromatography (SE-HPLC).

This white paper describes these methods and how they can be applied. To discuss any of the techniques or to find out more about how we can help you with any specific issue, contact:

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## Introduction

The chemical composition of raw ingredients affects their functionality. Understanding how raw ingredients function in food systems is critical to controlling final product quality. In addition to their nutritional value, proteins can confer many functional properties, including elasticity, extensibility, viscosity, foaming, gelling ability, coagulation, film forming ability and emulsification. Wheat proteins can form gluten, which gives dough its elasticity and extensibility, and consequently the characteristic texture and structure of the wheat-based products. Egg proteins have a range of different properties including foaming, coagulation and gelation, and are commonly used in a wide range of products such as cake, meringues and custard.

Proteins are composed of chains of amino acids (polypeptides) with a specific spatial conformation that corresponds with biological function. There are four levels of protein structure. Primary structure is the sequence of amino acids in the chain; individual amino acids are joined together by covalent peptide bonds. Secondary structure is the areas of folding and coiling within a protein and is determined by hydrogen bonds between the amino acids on the same chain. The overall three dimensional structure of a protein is its tertiary structure and is driven by non-covalent interactions. Quaternary structure refers to the non-covalent interactions that bind multiple polypeptides into a single, larger protein.

Proteins can be classified by their chemical structure, biological function or solubility. Proteins can have multiple functionalities in a food system, including structural, foaming, gelling, coagulating, film forming, emulsification and browning, as well as their nutritional role.

Different methods of analysis can reveal different characteristics of proteins – their overall structure, size, solubility and interactions – all of which can help predict functionality. Therefore, it is important to choose a method which will best give you the information you need.

## Polyacrylamide Gel Electrophoresis (PAGE)

Gel electrophoresis is a technique where proteins are separated according to their physical properties as they are forced through a polyacrylamide gel by an electric current. The gel matrix consists of a cross-linked polymer network which has a sieving effect. Different forms of this technique can be applied, which can provide different types of information, including composition, and evidence of denaturation or degradation. This information can be applied to track the effect of processing variables such as time, pH and temperature, and the use of processing aids such as reducing, oxidising or crosslinking agents and enzymes. This can help to determine which process is most appropriate. It can also help explain how a change in processing conditions has had a beneficial or deleterious effect on final product quality. For example, changing the pH of a formulation could lead to the presence or absence of haze (from suspended solids) – due to changes in protein solubility. Some commonly used PAGE techniques are described below.

*Native PAGE* separates proteins in their native state (intact quaternary structure) by their mass and charge. In general, the higher the negative charge density, the faster the protein will travel when the electric current is applied, and the sieving effect of the gel will retard larger molecules.

*SDS-PAGE (Sodium Dodecyl Sulphate-PAGE)* separates proteins mainly on the basis of their molecular weight. The ionic detergent (SDS) used in this method denatures (uncoils) the protein, and binds to it.

The SDS molecules have a negative charge, which masks the intrinsic charge of the protein. When an electric current is applied, the negatively charged proteins will migrate to the positive terminal at the bottom of the gel, with larger molecules travelling more slowly than smaller molecules. The position of the bands of proteins can be compared with standards of known molecular weight (see molecular weight markers in Figure 1). This analysis can be carried out in the presence or absence of a reducing agent. A reducing agent is used to break disulphide bonds between cysteine residues, resulting in complete separation of denatured proteins into their individual subunits. SDS-PAGE is useful for determining protein composition, and for determining whether denaturation and/or aggregation is occurring. In Figure 1, the effect of heating on egg white (A) and milk proteins (B) can be seen. These effects will have implications for the functionality of the proteins.

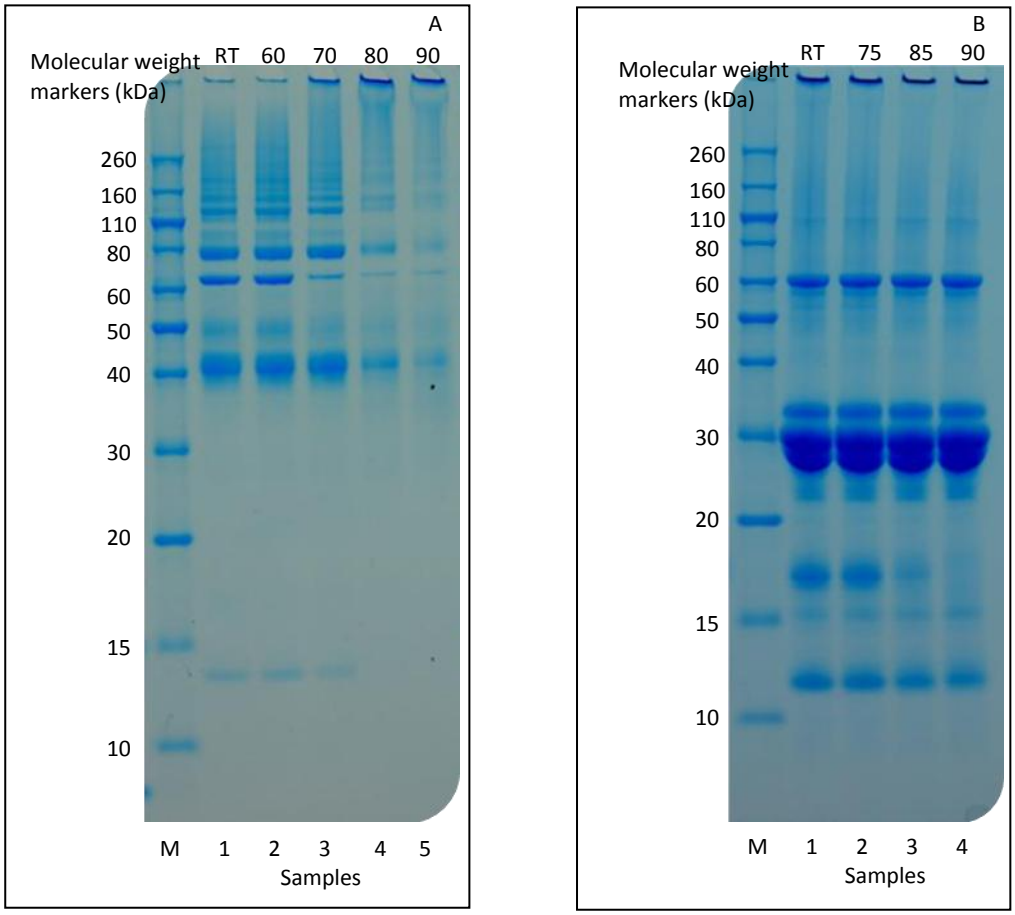


**Figure 1** SDS PAGE in non reducing conditions of A: Fresh egg white protein incubated at room temperature (RT), 60, 70, 80 or 90$^{o}$C for 20 minutes and B: Reconstituted skimmed milk incubated at room temperature, 75, 85 or 90$^{o}$C for 20 minutes.

*Isoelectric focussing*

Isoelectric focussing (IEF) separates proteins according to charge, and determines the point at which the net charge is zero (the isoelectric point, pI). Samples are loaded onto a gel with an established pH gradient. When the electric current is applied, the sample containing a mixture of proteins migrates through the pH gradient and individual proteins are immobilised in the pH gradient as they approach their pI. Proteins have minimum solubility in water or salt solutions at the pH that corresponds to their pI, and so often precipitate out of solution. Solubility is an important factor in determining protein functionality, and this technique can be used to help understand how changes in pH will affect the functionality of a product containing protein.

*Two dimensional PAGE* can resolve complex protein mixtures into individual protein spots. In this technique a sample is separated in the first dimension according to isoelectric point (IEF) and in the second dimension according to mass (SDS-PAGE).

**Size Exclusion High Performance Liquid Chromatography (SE-HPLC)**

SE-HPLC is a chromatographic method in which proteins in solution are separated by their size. This can help with understanding the root cause of problems in a product by examining differences in protein profile between a 'good' and 'bad' product. For example, it may be that quality of a product in which a protein-degrading enzyme is used has unexpectedly deteriorated. SE-HPLC can be used to compare the size profiles of the proteins from the products. It can also be used to compare raw materials with different properties.

Proteins are extracted from samples with detergent and mild sonication, meaning that many associations between protein molecules are preserved. Separation is usually achieved using a column tightly packed with extremely small porous polymer beads designed to have pores of different sizes. As the proteins in solution travels down the column, some particles enter into the pores. Larger particles cannot enter into as many pores as smaller ones, and so the larger particles travel faster through the column and exit first.

For proteins that form large complexes, such as wheat proteins, SE-HPLC allows changes in the size of the macromolecule to be observed. The size of complex that can be analysed using this method ranges from approximately 700kDa to 10kDa. This would not be possible with native PAGE. Results from this analysis can be related to functional properties such as bread making quality.

In the example below, the effect of processing such as adding oxidising or reducing agents (Figure 2), or the effect of mixing (Figure 3) on a wheat flour dough can be examined. An oxidising agent promotes formation of bonds between proteins, leading to an increase in the proportion of larger molecules, while reducing agents break bonds and have the opposite effect.

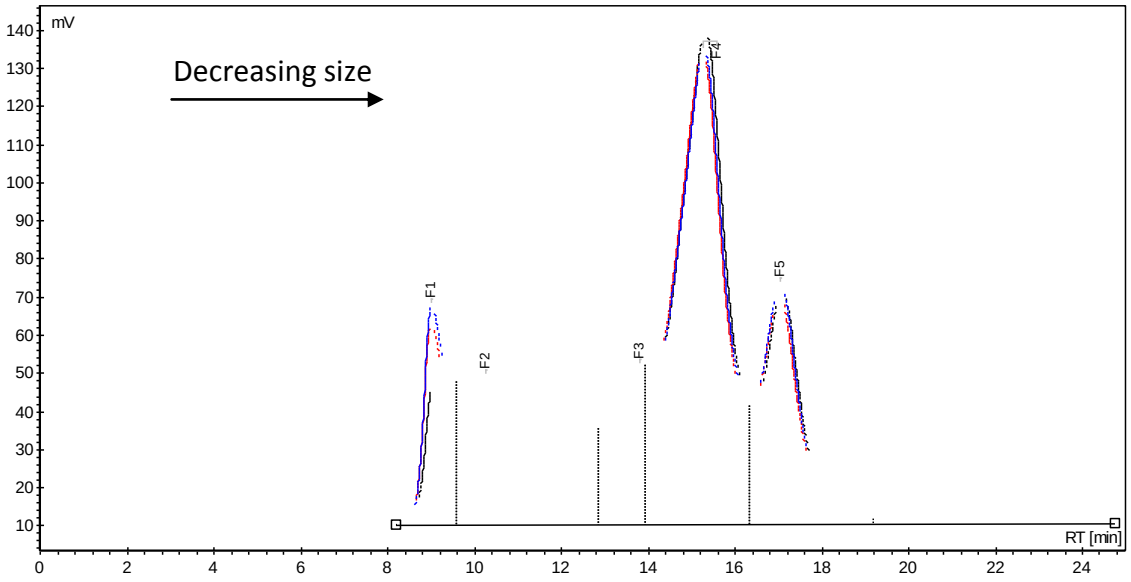


**Figure 2** Effect of oxidising (blue) and reducing (black) agents on dough proteins (control red). Samples extracted with sonication.

In Figure 3 below, mixing was shown to have the effect of increasing the proportion of larger, soluble proteins – again a characteristic shown up by SE-HPLC.

Varying the extraction technique by using different degrees of sonication, can give different information about a sample.

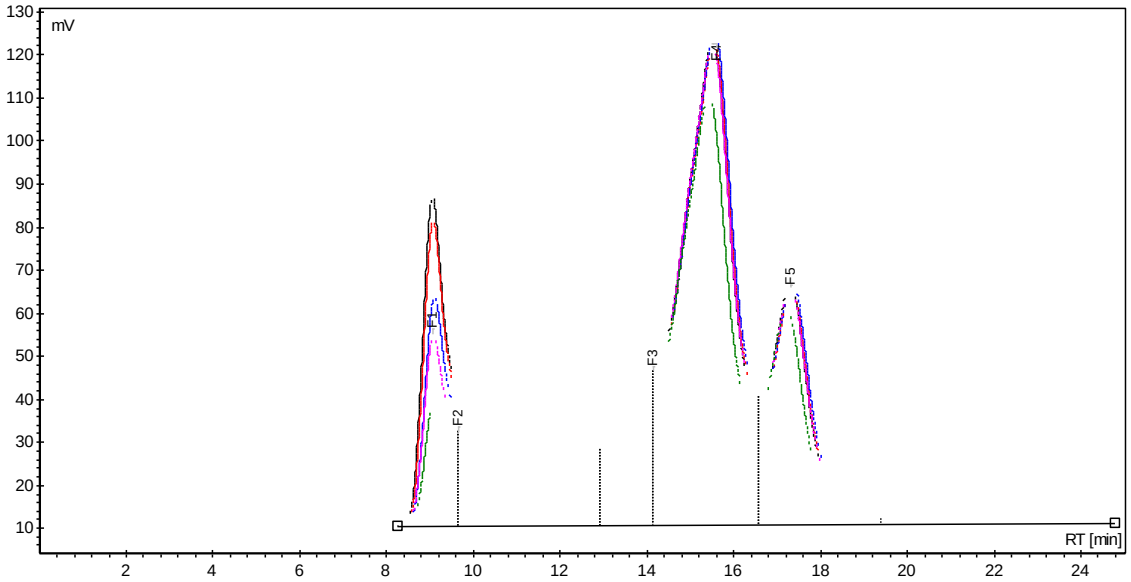


**Figure 3** Effect of mixing time (0 (flour; green), 1 (pink), 2 (blue), 5 (red) and 10 (black) minutes) on dough proteins. Samples extracted without sonication.

**Contact us**

Protein analysis provides a versatile suite of techniques for understanding proteins in food stuffs. Different methods will be applicable, depending on what you need to know. Please contact us if you think we can help you.

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