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## DEPARTMENT OF FOOD TECHNOLOGY

### 23FTT204- BIOCHEMISTRY & NUTRITION

#### UNIT 3- PROTEINS

#### TOPIC 4 & 5,6 - Properties of proteins in food systems: solubility, hydration, foam formation & stabilization, gel formation, emulsifying effect. Denaturation. Solubility

The functional properties of protein are often affected by protein solubility and these most affected are thickening, foaming, emulsifying and gelling. Insoluble protein has very limited uses in food. The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein –protein and protein solvent interaction.

#### **Protein- protein + solvent – solvent = protein –solvent**

The major interactions that influence the solubility characteristics of protein are hydrophobic and ionic in nature. Hydrophobic interaction promote protein –protein interaction and result in decreased solubility, whereas ionic interaction promote protein –water interactions and result in increased solubility. Ionic residues introduce two kinds of repulsive forces between protein molecules in solution. The first involves electrostatic repulsion between protein molecules owing to a net positive or negative charge at any pH other than the isoelectric pH, the second involves repulsion between hydration shells around ionic groups. Based on solubility characteristics, proteins are classified into four categories. Albumins are those that are soluble in water at pH 6.6 (e.g. Serum albumin, ovalbumin & - lactalbumin), globulins are those that are soluble only in acid (pH 2) and alkaline (pH 12) solutions (e.g. wheat glutins) and prolamins are those soluble in 70% ethanol (e.g. zein and gliadins ). Both prolamines and glutelins are highly hydrophobic proteins. In addition to these intrinsic physiochemical properties, solubility is influenced by several solution conditions, such as pH ionic strength, temperature and the presence of organic solvents, protein source, processing history, minor and major treatments in its preparation & processing , heating, protein concentration and the presence of other ingredients.

At constant pH & ionic strength, the solubility of most proteins generally increases with temperature between 0 and 40 °C. Exceptions occur with highly hydrophobic proteins, such as B- casein & some cereal protein, which exhibit a negative relationship.

## **Protein hydration**

Water is an essential constituent of foods. The rheological & textural properties of depend on the interaction of water with other food constituents, especially with macromolecules, such as proteins and polysaccharides. Water modifies the physiochemical properties of proteins. For example, the plasticizing changes their glass transition temperature and TD. Many functional properties of protein such as dispersibility, wettability, swelling, solubility, thickening, viscosity, water holding capacity, gelation, coagulation, emulsification and foaming depend on water protein interactions, the ability of protein to bind water is critical to the acceptability to these foods. Water molecules bind to several groups proteins. These include charged groups / ionic dipole interaction), backbone peptide groups, the amide groups of Asn & Gln, hydroxyl groups of Ser, Thr, and Tyr residues (all dipole-dipole interactions) and nonpolar residues (dipole induced dipole interaction, hydrophobic hydration). Several environmental factors, such as pH, ionic strength, type of salts, temperature and protein conformation, influence the water binding capacity of proteins. Protein exhibit the least hydration at their isoelectric pH, where enhanced protein interactions results in minimal interaction with water. Above & below the isoelectric pH, because of the increase in the net charge & repulsive forces, protein swells and binds more water. The water binding capacity of most protein is greater at pH 9-10 than at any other pH. This is due to ionization of sulfhydryl & tyrosine residues. Above pH 10, the loss of positively charged  $\alpha$ -amino groups of lysyl residues result in residues water binding. At low concentration (<0.2) salts increase the water binding capacity of proteins. This is because hydrated salt ions bind (weakly) to charged groups on proteins. The water binding capacity of protein generally decrease as the temperature is raised, because of decreased hydrogen bonding & decrease hydration of ionic groups. The water binding capacity of a denatured protein is generally about 10% greater than that of the native protein. This is due to an increase in surface area to mass ratio with exposure of some previously buried hydrophobic groups. However, if denaturation leads to aggregation of the protein, then its water binding capacity may actually decrease because of the protein-protein interactions. The solubility of a protein is dependent not only on water binding capacity, but also on other thermodynamic factors. In food applications, the water holding capacity of a protein preparation is more important than the water binding capacity refers to the ability of the protein to imbibe water & retain it against gravitational force within a protein matrix, such as protein gels or beef and fish muscle. This water refers to the physically entrapped water. The contribution of the physically entrapped water to water holding capacity is much larger than those of the bound textural properties of bakery and other gel type products.

## **Gelation**

A gel is an intermediate phase between a solid a liquid. Technically, it is defined as a substantially diluted system which exhibits no steady state flow. Protein gels are composed of three dimensional matrices or networks of intertwined, partially associated, polypeptides, in which water is entrapped. Gels are characterised by a relative high viscosity plasticity & elasticity. Protein gelation refers to transformation of a protein from the sol state to a gel like state. This transformation is facilitated by heat, enzymes or divalent cations under appropriate conditions.

Typically protein gels are gelation gelly coagulated egg white, soybean toffee, milk casein curds & the myofibrillar gels by heating saline soluble meat or fish protein. The ability of

protein to form a gel & provide a structurally matrix for holding water, flavours, sugars & food ingredients is useful in food application and gelation process would be greatly accelerated compared to the first step in new product development because it provides an added dimension to protein functionality. Protein gel formation usually requires prior heating of a protein to cause at least partial denaturation or unfolding of the polypeptide chains. Gelation is a two-stage process involving the initial denaturation of native protein into unfolded polypeptides, which then gradually associated to form the gel matrix if attractive forces and thermodynamics condition is suitable. Because of the high temperature coefficients of denaturation it may be expected that the first step in the gelation process would be greatly accelerated compared to the second step, involving network formation, therefore higher temperature should result in the formation of finer firmer gels. However, condition of the cooling steps, which is usually required to permit gelation also affect physical characteristics of gels. Upon cooling, the uncoiled polypeptides associated to form the network. Cross linking may involve multiple hydrogen bonds ionic attraction disulfide bonds, hydrophobic association or a combination of these and types vary quantitatively and qualitatively with different protein gels. The stability of a gel network against thermal and mechanical forces is dependent on the no. of type of cross links formed per monomer chains. This is dependent on several intrinsic (such as the size, net charge etc.) & extrinsic factors (such as pH, temperature ionic strength etc.)

### **Foaming properties**

Foaming or whipping i.e. the capacity to form stable foams with air is an important functionality of protein in several products viz. Angel food cakes, sponge cakes, divinity type confection, candy meringue, soufflés, varies whipped topping, icings, fudges, nougats etc. Foaming properties included whip ability and foam ability. These properties are measured as foam expansion, foam capacity or overrun all of which essentially refer to the maximum volume increase of a protein of a protein dispersion, following the incorporation of air, by whipping, agitation or aeration. Foaming power measure the measures the increase in volume, upon the introduction of a gas into a protein solution or dispersion, foam stability refers to the ability of a formed foam to retain its maximum volume over time, & it is usually determined by measuring the rate of leakage of fluid from the foam. Foam stability is determined by measuring loss of fluid resulting from destabilization i.e. leakage, measuring volume decrease or density increase with time.

### **Emulsifying & Foaming properties**

Emulsions are disperse systems of one or more immiscible liquids. They are stabilized by emulsifiers – compounds which form interface films and thus prevent the disperse phases from flowing together.

Proteins are amphiphilic molecules, and they migrate spontaneously to an air-water interface or an oil-water interface. This spontaneous migration of proteins from a bulk liquid to an interface indicates that the free energy of proteins is lower at the interface than it is in the bulk aqueous phase.

Protein-stabilized foams and emulsions are more stable than those prepared with low-molecular-weight surfactants, and because of this, proteins are extensively used in food applications.

Although all proteins are amphiphilic, they differ significantly in their surface-active properties. The differences in surface activity are related primarily to differences in protein conformation. The conformational factors of importance include stability/flexibility of the polypeptide chain, ease of adaptability to changes in the environment, and distribution pattern of hydrophilic and hydrophobic groups on the protein surface. All of these conformational factors are inter-dependent, and they collectively have a large influence on the surface activity of proteins.

Desirable surface-active proteins should have three attributes:

- (a) Ability to rapidly adsorb to an interface,
- (b) Ability to rapidly unfold and reorient at an interface, and
- (c) Ability to interact with the neighboring molecules and form a strong cohesive, viscoelastic film that can withstand thermal and mechanical motions.

Functional role in foods

Several natural and processed foods, such as milk, egg yolk, coconut milk, soy milk, butter, margarine, mayonnaise, spreads, salad dressings, frozen desserts, frankfurter, sausage, and cakes, are emulsion-type products where proteins play an important role as an emulsifier.

In natural milk, the fat globules are stabilized by a membrane composed of lipoproteins. When milk is homogenized, the lipoprotein membrane is replaced by a protein film comprised of casein micelles and whey proteins.

Homogenized milk is more stable against creaming than is natural milk because the casein micelle-whey protein film is stronger than the natural lipoprotein membrane.

The emulsifying properties of food proteins are evaluated by several methods such as size distribution of oil droplets formed, emulsifying activity, emulsion capacity, and emulsion stability. The properties of protein-stabilized emulsions are affected by several factors.

These include intrinsic factors & extrinsic factors.

**Intrinsic factors:** These include pH, ionic strength, temperature, presence of low-molecular-weight surfactants, sugars, oil-phase volume, type of protein, and the melting point of the oil used.

**Extrinsic factors:** These include type of equipment, rate of energy input, and rate of shear.

### **Denaturation:**

Denaturation is a phenomenon that involves transformation of a well-defined, folded structure of a protein, formed under physiological conditions, to an unfolded state under non-physiological conditions.

The native structure of a protein is the net result of various attractive and repulsive interactions emanating from various intramolecular forces as well as interaction of various protein groups with surrounding solvent water. However, native structure is largely the product of the protein's environment. Any change in its environment, such as pH, ionic strength, temperature, solvent composition, etc., will force the molecule to assume a new equilibrium structure. Subtle

changes in structure, which do not drastically alter the molecular architecture of the protein, are usually regarded as “conformational adaptability,” whereas major changes in the secondary, tertiary, and quaternary structures without cleavage of backbone peptide bonds are regarded as “denaturation.”

Many biologically active proteins lose their activity upon denaturation. In the case of food proteins, denaturation usually causes insolubilization and loss of some functional properties. In some instances, however, protein denaturation is desirable. Example :Thermal denaturation of trypsin inhibitors in legumes markedly improves digestibility and biological availability of legume proteins when consumed by some animal species.

Partially denatured proteins are more digestible and have better foaming and emulsifying properties than native proteins. Thermal denaturation is also a prerequisite for heat-induced gelation of food proteins.

□ Amphotericism: The ability of a chemical to behave both as an acid or base is called amphotericism & the substance is called amphoteric substance. These substances act as acids in presence of base &viceversa.

Examples of amphoteric substances include water, amino acids, and proteins. Proteins are amphoteric polyelectrolytes, i.e. they possess both acidic and basic properties. The acid-basic properties of amino acids are primary due to occurrence of  $\alpha$ -amino and  $\alpha$ -carboxyl groups (i.e. acid-base pairs) in them. The amphotericism of proteins is due to the acid-base groups of side-chain radicals of protein-constituting amino acids.