Revisión

Bol. Soc. Quím. Méx. **2008**, *2(2), 80-89*

ISSN 1870-1809

Emulsifying properties of proteins

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Abstract: Emulsions form the basis of a huge range of food products, where those stabilized by proteins are of great interest. The interfacial properties of proteins have been extensively studied in the field of food colloid research. Emulsifying properties of proteins basically depend on two effects: (1) a substantial decrease in the interfacial tension due to the adsorption of the protein at the oil-water interface and (2) the electrostatic, structural and mechanical energy barrier caused by the interfacial layer that opposes the destabilization processes. The knowledge on the biochemical and physicochemical characteristics, interfacial behaviors and emulsifying properties of proteins will help us understand the structure-function relationship of the emulsifying proteins.
Key words: Proteins, Food Emulsions, emulsifying properties, protein functionality, interface.

Resumen: Muchos productos alimenticios son emulsiones, de las cuales aquellas estabilizadas por proteínas son de gran interés. Las propiedades interfaciales de las proteínas han sido ampliamente estudiadas en el campo de la investigación de los coloides alimentarios. Las propiedades emulsificantes de las proteínas dependen básicamente de dos efectos: (1) un decremento sustancial de la tensión interfacial debido a la adsorción de la proteína en la interfase aceite-agua y (2) la barrera de energía electrostática, estructural y mecánica causada por la capa interfacial que se opone a los procesos de desestabilización. El conocimiento de las propiedades bioquímicas y fisicoquímicas, del comportamiento interfacial y de las propiedades emulsificantes de las proteínas ayudará a entender la relación estructura-función de las proteínas emulsificantes.
Palabras clave: Proteínas, emulsiones alimenticias, propiedades emulsificantes, funcionalidad de proteínas, interfase.

Introduction

Proteins are essential ingredients in the food industry, not only due to their nutritive value, but because of all the other properties, known as "functional properties" [1]. These properties result form the physicochemical interactions among the food system components; the most widely used are the interfacial ones [2] such as foaming and emulsifying properties, which are used in the food industry. Proteins are the single most commonly used ingredients since they are natural, non toxic, cheap and widely available, thus making them ideal ingredients.

Food Emulsions

Food emulsions are defined on a molecular level as complex colloidal systems comprising two immiscible phases, one dispersed in the other. The study of emulsions is complicated by the interactions that can occur when multiple components are present; the fact is that the systems are easier to study in dilute solutions, under conditions that may not apply to those likely to be found in foods. It is possible to explain how proteins work in emulsions from the knowledge of the forces that operate during emulsion formation and from information about protein structure [2-4].

The intrusion of a non-polar molecule interferes with the normal structure of water increasing its order [5]. When a liquid of low polarity such as fat is mixed with water there is a strong driving force to limit the contact between the two liquids. This happens when phase separation occurs. When two immiscible liquids are forced into contact by the application of work, the result will be the formation of a number of spherical droplets within the dispersed phase. Given enough time this leads to the situation of minimum contact and phase separation [6].

Recently, the interest in microemulsions and nanoemulsions has grown. Microemulsions are thermodynamically stable, and transparent, with a low viscosity and isotropic dispersions consisting of oil and water stabilized by an interfacial film of surfactant molecules, typically in conjunction with a cosurfactant. Microemulsions (so-called due to their small particle size; 50-1000 nm) can be applied in a wide variety of systems, such as pharmaceutical processes [7,8] and oil recovery, but their application in food systems has been hindered by the types of surfactant permissible in the food industry. Nanoemulsions can be defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging from 5 to 100 nm. Usually, the average droplet size is between 100 and 500 nm. The terms sub-micron emulsion (SME) and mini-emulsion are used as synonyms. Emulsions which match this definition have been used in parenteral nutrition for a long time. Usually, SMEs contain 10 to 20 per cent oil stabilized with 0.5 to 2 per cent emulsifying protein or peptide [9].

a) Mechanisms of Emulsion Stability

The disperse system can be stabilized against coalescence and phase separation if another component, partially soluble in both phases, is added. Molecules that are composed of portions that are soluble in water and portions that are soluble in lipids can serve as emulsifiers. An emulsion formed from a mixture of oil, water and emulsifier is at a higher energy level than the non emulsified system. The goal of the food scientist

is to elevate the energy of activation in order to give the emulsion a reasonable lifetime [6].

In order to form an emulsion, energy must be provided in excess, due to the creation of the new interfacial area of the emulsion. The size of the droplets and thus their interfacial energy depends on the amount of work done on the system. The rate of coalescence depends on the energy barrier and the rate of droplet collision

b) Emulsion destabilization

Once an emulsion is formed, it can undergo several changes. With protein stabilized emulsions, phase inversion is generally not a problem because when fat globules approach each other, the proteins usually provide an effective barrier to coalescence. The removal of proteins from the surface of a fat globule is energetically unfavourable and does not occur at any appreciable rate. In food products, fluctuations in temperature are a common cause of emulsion destabilization. As the temperature is lowered, water attains more and more structure. As the water becomes more ordered, there is a lower energy difference between the hydrophobic groups exposed to the aqueous phase and those buried in the oil phase. When the system is thawed, coalescence occurs when the physical damage has been extensive. One of the best ways to minimize this type of damage is by adding substances that will modify the size and extent of water crystal formation [11].

A more common defect in food emulsions results from the phenomenon known as creaming. If enough time is given or a centrifuge force is applied a depletion of the lipid from the bulk aqueous phase occurs with the formation of a compact cream layer containing the majority of the lipid (**Figure 1**). The extent of the emulsion shelf life depends on the fact that the density of the fat globules must be made identical to that of the continuous phase or the viscosity must be high enough so that the yield value is greater than the acceleration due to buoyant differences.

Low-molecular weight emulsifiers are often more surface-active than proteins, and will therefore compete for interfacial area (competitive adsorption). It is known that adding surfactants to protein-stabilised emulsions will have a detrimental effect on stability [12,15,16]. In fact, surfactants are sometimes used as the anti-emulsifying agents [12]. Surfactants are known to displace proteins from emulsion droplets [17,18]. It has been shown that the addition of polysaccharide stabilizers to emulsions has little effect on the stability of the systems unless they increased the viscosity to the point of imparting a yield value. [19]. Thus, while Stoke's law is important in predicting the rate of emulsion creaming, for most products with any appreciable shelf life, other factors, especially viscosity, pseudoplasticity and yield stress, must also be considered [20,21].

The amount of damage done to a product by the formation of a cream layer depends on the product type and the tenacity of the formed layer [22].

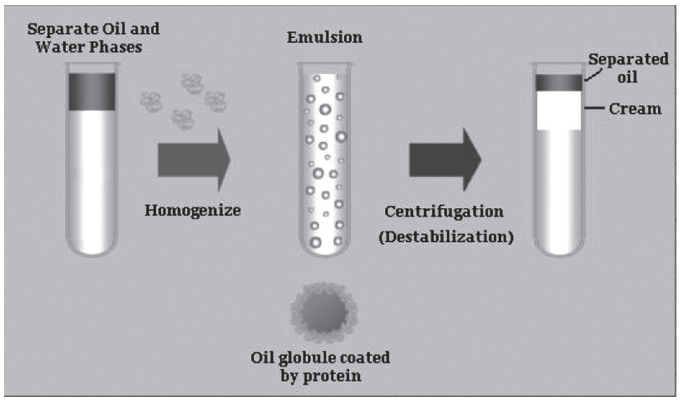

Fig. 1 Emulsion destabilization to measure the emulsion stability by appliance of centrifuge force.

c) Surfactants classification

Emulsifiers can be divided into two categories:

- Small molecules: Mono and diglicerides, Sucrose Esters, Sorbitan Esters (SPAN), Polysorbates (TWEEN), Stearoyl Lactylates, Lecithin and Derivatives
- Macromolecules: Proteins such as bovine serum albumin, b-lactoglobulin, lysozyme, and ovalbumin

Only food emulsifiers defined as food additives are usable by law. Those emulsifiers are shown in **Table 1** which had been used ordinarily in food systems.

Proteins as Emulsifiers

Protein functionality has been defined as: «any property of a protein, exception being its nutritional ones that affects its utilization» [23]. Proteins show a large number of functions and functional properties and some of the most important are shown on **Table 2**.

Protein functionality is evident by its interaction with other components within the food or chemical system. These interactions may involve solvent molecules, solute molecules, other protein molecules or substances that are dispersed in the solvent such as oil or air [24-26].

When proteins are used to generate emulsions, the system becomes highly complex. They are responsible for the creation of a new surface area. The high energy state is relieved by rapid coalescence of fat globules. For prevention of coalescence, protein molecules need to diffuse to the fat/ water interface and then unfold and coat the surface. When enough of the new surface is covered, coalescence ceases. With proteins, the rate of diffusion to the interface is a significant variable in the amount of protein that absorbs to the interface during the emulsion formation. If something tends to decrease the rate of diffusion of the protein molecules, the protein load decreases [6].

Table 1. Food Emulsifiers utilized in Food Systems[a]

Name	Common Name
Glycerin Fatty Acid Esters	Monoglyceride (MG)
Acetic Acid Esters of Monoglycerides	Acetylated Monoglyceride (AMG)
Lactic Acid Esters of Monoglycerides	Lactylated Monoglyceride (LMG)
Citric Acid Esters of Monoglycerides	CMG
Succinic Acid Esters of Monoglycerides	SMG
Diacetyl Tartaric Acid Esters of Monoglycerides	DATEM
Polyglycerol Esters of Fatty Acids	PolyGlycerol Ester (PGE)
Polyglycerol Polyricinoleate	PGPR
Sorbitan Esters of Fatty Acids	Sorbitan Ester (SOE)
Propylene Glycol of Fatty Acids	PG Ester (PGME)
Sucrose Esters of Fatty Acids	Sugar Ester (SE)
Calcium Stearoyl Di Laciate	CSL
Lecithin	Lecithin (LC)
Enzyme Digested/Treated Lecithin	EDL or ETL
Proteins	

[a] http://www.rike-vita.co.jp/int/emulsifier/basic/LMG#LMG

Table 2. Typical Functional Properties performed by proteins in Food Systems[a]

Functional Property	Mode of action	Food system
Solubility	Protein solvation, pH dependent	Beverages
Water absorption and binding	Hydrogen-bonding of HOH, entrapment of HOH (no drip)	Meats, sausages, breads, cakes
Viscosity	Thickening, HOH binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds, cheese
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products
Elasticity	Hydrophobic bonding in gluten, disulfide links in gels (deformable)	Meats, bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup, cakes
Fat adsorption	Binding of free fat	Meats, sausages, donuts
Flavor binding	Adsorption, entrapment, release	Simulated meats, bakery, etc.
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon desserts, angel cakes

[a] From Kinsella and Srinivasan [44].

Emulsions are thermodynamically unstable mixtures of immiscible liquids. If energy is applied the systems may be dispersed, but an increment on the surface energy causes the phases to coalesce unless an energy barrier that would prevent coalescence is established. Emulsified droplets can be stabilized by the addition of molecules that are partially soluble in both phases. In foods a number of small emulsifier molecules can serve this function. Proteins capable of unfolding at the interface may also serve this function. Protein coats the lipid droplet and provides an energy barrier to particle association and phase separation [27-29].

Proteins are included in emulsions to aid in their formation and to increase their stability. They are much larger and more complex than other simple emulsifier molecules and the formation of a protein stabilized emulsion requires that the protein molecule must first reach the water/ lipid interface and then unfold so that its hydrophobic groups can contact the lipid phase. To illustrate the forces involved, the situation of a protein molecule approaching a static water/lipid interface will first be considered. In native proteins most of the non-polar amino acid side chains are located in the interior of the molecules. Proteins have charged groups at the surface of the molecule which are in contact with water molecules. The favourable interaction of water with surface charge lowers the total energy of the protein molecule. The hydrophobic groups are removed from contact with the aqueous phase while charged groups maximize solvent contacts [6,11].

As a protein molecule approaches the interface, there is less opportunity for the charged groups to interact with the solvent. In the extreme case, charged groups are removed from the aqueous phase and enter the lipid phase. This is energetically unfavourable and these groups are repelled from the interfacial area. If the groups closer to the interface are in a region of the protein molecule that contains some flexibility,

the molecule may begin to unfold. This unfolding causes the exposure of hydrophobic groups to the surface. If these groups are exposed to the aqueous environment, there is an increase in total energy and random fluctuations in protein structure cause these groups to return to the inner part of the molecule. If the exposure occurs at an interface, the state of lowest free energy depends on the nature of the interface. In the case of a protein un-folding near lipid, the hydrophobic groups are inserted into the lipid phase. This insertion has a very low energy of activation and proceeds spontaneously. For proteins such as soybean glycinin [30-32], tryptophan synthase [33] and lysozyme [3,34,35], the size of the hydrophobic region inserted is about 6 to 8 amino acid residues. The enthalpy for this step is positive so that the driving force must be an increment in the entropy of the system. This increase in entropy has two components, one due to the conformational entropy of the protein and one due to the structure of water near hydrophobic groups. There is an increment in the conformational entropy of the protein as the hydrophobic groups are removed from the interior of the molecule and placed into another non-polar environment. The original protein had a limited number of ways of arranging its components to attain a low energy state. The partially unfolded molecule has many ways of inserting a hydrophobic group into a non-polar environment and once there the group can assume more conformations than before. The solvent molecules at the interface are arranged in highly ordered structures. The protein with hydrophobic groups inserted will coat the non-polar material and will release the solvent from the surface. The release of this water is responsible for a significant increase in the entropy of the system [11].

While the original insertion of a hydrophobic group proceeds spontaneously with a small energy of activation, the reaction is not readily reversible. In time other sections of the protein molecule approach the surface and if these occur in flexible portions of the protein they too may be inserted into the lipid phase. As this continues the protein will unfold at the interface [6].

Proteins that become attached by more than one hydrophobic group desorb very slowly from the surface, if at all. Langmuir and Schaeffer [36] calculated that if absorption were completely reversible and the Gibb's absorption equation is applied, the changes in the magnitude of surface pressure they observed, in ovalbumin stabilized emulsions, resulted in an essentially complete desorption of protein form the interface. This does not occur for protein stabilized emulsions suggesting that a significant energy barrier to protein desorption exists. Removal of hydrophobic groups from the lipid exposes the lipid to the aqueous phase as well as the hydrophobic groups that are being removed. Even if the removed hydrophobic groups could be buried in the protein interior, the protein would remain attached to the fat globule at other points and reattachment would be likely. If other hydrophobic molecules are available to cover the exposed lipid area, desorption is easier to achieve. It has been shown, for instance, that gelatin molecules can be replaced by more hydrophobic casein molecules from the water/ lipid interface [29,37].

Once a layer of protein has been adsorbed additional protein layers cannot be added in the same way since an energy barrier to absorption arises. In order for more protein to be absorbed, the protein already at the surface must be compressed to make room. The amount of compression that is possible depends on the rigidity of the protein and also on the amount of residual charge near the surface. At some level of compression, the absorption of more protein will require more energy, which can be gained by the insertion of hydrophobic groups into the lipid layer. Further interaction involves the interaction of protein molecules in the bulk phase with those already adsorbed to the lipid and the formation of multilayer [37].

a) Classification of Protein Based Surfactants

The three types of protein-based surfactants are: (1) Amino Acids, (2) Peptides, both of them derived from synthesis and hydrolysis of the (3) Proteins [38].

The amino acid-based surfactants are composed of an amino acid as the hydrophilic part and a long hydrocarbon chain as the hydrophobic part. The hydrophobic chain can be introduced through acyl, ester, amide, or alkyl linkage [39]. Examples of these kind of surfactants are the long-chain N^a-acyl amino acid derivatives from pure amino acids or protein hydrolysated, which have been extensively used in the cotton chemical industry [40]. *N*-Acylsarcosinate salts are suitable for cosmetics, toothpaste, wound cleaners, personal care items, shampoo, bubble-bath pastes, aerosols and synthetic bars [41]. The many kinds of amino acid-based surfactants have a potential wide application in the cosmetic, personal care, food, and drug industries.

The peptide surfactants are derived from the condensation of dipeptides or tripeptides and hydrophobic chains such as fatty acids. Most of the surfactants in literature have been chemically synthesized, although some have been biosynthetically produced [42]. Examples of these surfactants are the diethanolamides (DEA) of *N*-lauroyl dipeptides of various molecular structures [43].

b) Molecular basis of protein surfactants

It is essential that the forces and energies involved in the achievement and maintenance of native protein structure be described. While a complete discussion of the forces involved is beyond the scope of this revision, some observations on the nature of protein structure will be useful.

Hydrophobic Interactions. One of the main mechanisms by which proteins diminish their free energy involves the removal of hydrophobic groups from the aqueous environment. This may provide the greatest single decrease in free energy of all the types of binding that occur within proteins [2,5,33,44]. The strength of hydrophobic binding is, however, very sensitive to

changes in temperature and the dielectric constant, thus, the changes in these parameters strongly influences protein structure [45].

Once a protein begins to unfold, there must be hydrophobic groups present to insert into the non-polar phase. In theory, a measure of the relative hydrophobicity of a protein should be related to its ability to function as an emulsifying agent [3,46]. In practice, relative hydrophobicity measurements have been difficult to obtain [47]. The early methods generally assigned some relative value to each amino acid and then the value for the protein is calculated from its composition [48-49]. These procedures have rarely correlated well with functionality because they measure the total potential of hydrophobicity of the protein rather than those of the hydrophobic groups which can actually reach the surface upon unfolding. Recently a number of procedures have been developed which measure what is termed the "effective hydrophobicity" of proteins, which means obtaining a quantitative measure of those hydrophobic groups that are capable of binding to a selected probe molecule. The groups that are deeply buried in a portion of the protein that does not unfold are not measured, while those accessible to the protein surface are the ones detected by the probes (fluorescent) and the ones able to interact in emulsions or foams. The quantum yields of fluoresce and wavelength of maximum fluorescence emission of these compounds depend on the polarity of their environment [50]. Due to high sensitivity, non-invasiveness, and availability of imaging techniques, fluorescence spectroscopy has been considered to be one of the most promising and potentially widely used techniques in medicine, biology, biochemistry, and molecular biophysics for the 21st century [51-52]. Fluorescent probes used include 1-anilinonaphthalene-8-sulfonic acid (ANS), *cis*-parinaric acid (CPA) and 6-propionyl-2-(dimethylamino)-naphthalene (PRODAN) have been widely used to measure protein hydrophobicity. These probes have a low quantum yield of fluorescence in aqueous solution. Upon binding of the probes to accessible hydrophobic regions of proteins, an increase in fluorescence is observed, which is used as a measure of protein surface hydrophobicity. However, due to the possible contribution of both electrostatic and hydrophobic interactions to the binding of these anionic probes, the interpretation based on these probes has not been easy. Therefore, an uncharged probe is needed to circumvent this problem [53].

The distribution of hydrophobic groups is also important. In proteins such as ß-lactoglobulin [54], the hydrophobic groups are evenly distributed throughout the molecule. There are no large portions of the molecule where hydrophobic amino acids are grouped, nor are there large sections of the molecule that do not contain charged amino acids [55]. This makes it difficult to find portions of the molecule that are sufficiently hydrophobic or to find residues that do not contain amino acids with charged groups that would resist their removal from the aqueous phase. In molecules such as b-casein there are large sections of the protein that contain hydrophobic amino acids without the presence of charged groups. The molecule has such an uneven distribution of charge and hydrophobic groups that it is amphipathic. It is easy to find portions of this molecule that contain at least six non-polar amino acids and no charged groups [56].

Electrostatic Interactions. Electrostatic interactions play a major role in the determination of the molecular structure of a protein [57]. Proteins contain a number of amino acids that can ionize to form either positively charged ions (e. g., arginine, lysine, proline, histidine and the terminal amino group) or negatively charged ions (e. g. glutamic and aspartic acids and the terminal carboxyl group) [57-58]. If the protein contains many similarly charged groups, it is more likely to adopt an extended configuration because this increases the average distance between the charges and therefore minimizes the unfavourable electrostatic repulsions. If, on the other hand, the protein contains many oppositely charged groups, it is more likely to fold up into a compact structure that maximizes the favourable electrostatic attractions. As a result, proteins are often extremely compact at their isoelectric point and unfold as the pH is either increased or decreased. Electrostatic interactions also play an important role in determining the aggregation of proteins in solution. Similarly charged proteins repel each other and therefore tend to exist as individual molecules, whereas oppositely charged proteins attract each other and therefore tend to aggregate (depending of the strength of the various other types of interactions involved). The binding of low-molecular weight ions, such as Na^{+} and Ca^{2+}, is also governed by electrostatic interactions and may influence the strength of the hydration repulsion between proteins in solution [59].

Hydrogen Bonding. Proteins contain monomers that are capable of forming hydrogen bonds. Hydrogen bonds are a relatively strong type of molecular interaction, and therefore a system attempts to maximize the number and strength of the hydrogen bonds formed. The protein may adopt an arrangement that enables it to maximize the number of hydrogen bonds which are formed between the monomers within it, which leads to the formation of ordered regions such as helixes, sheets and turns [58]. Alternatively, a protein may adopt a less ordered structure where the monomers form hydrogen bonds with the surrounding water molecules. Thus, a part or all of the protein may be found in either a highly ordered conformation (which is entropically unfavourable) with extensive intramolecular hydrogen bonding or in a more random-coil conformation (which is entropically more favourable) with extensive intermolecular hydrogen bonding. The type of structure formed by a protein under certain sets of environmental conditions is governed by the relative magnitude of the hydrogen bonds compared to the various other types of interactions, most notably hydrophobic, electrostatic and configurational entropy [59].

Configurational Entropy. Proteins exist in the lowest kinetically attainable state of free energy. The free energy of the protein may not be the global minimum, but it will be the lowest that the protein can achieve in a reasonable period of time. Protein structure is highly dependent upon the environment and

the protein will assume different conformations as the environmental conditions change. Factors of importance include pH, temperature, dielectric constant, ionic strength and the presence of other molecules including air, fat, denaturants, etc.

The structures attained by proteins are not rigid but very dynamic. There is rotational freedom around many of the bonds within the protein molecule and the entropy gain of this freedom lowers the total free energy of the native structure. There are also portions of the protein structure that are stabilized by rather weak secondary forces and these are often free to assume different conformations. These alternate conformations lead to structures of higher free energy and thus are not stable or long lived. A protein may be envisioned as a dynamic entity that is constantly trying a variety of structures. These new structures are usually only slightly different from the native conformation and almost always lead to a situation where the free energy of this system increases. The increment in free energy causes the protein to spontaneously refold into the state of lowest free energy. Thus, the native structure of a protein is not the only structure it can assume, but rather the one with the lowest free energy and hence the greatest probability [59-62]. Slight changes in the environment can cause alternate structures to be of a lower free energy and thus lead to protein denaturation. In order for a protein to exhibit functionality, it must interact with other components of the food system. These interactions may often require that the protein be free to either move throughout the system or to alter its structure in such a way to allow interactions with other components. In some cases the simple presence of other molecules in the protein solution will allow interaction to occur, but more commonly, the interactions require an input of energy into the system to insure adequate mixing. This energy may alter the physical nature of the molecules being mixed, e.g. decrease in average fat globule size, and also alter the conformation of the protein molecule [21, 63,64].

Disulfide Bonds and Protein Flexibility. Flexibility is an important feature affecting the emulsifying properties of proteins [33,65]. In aqueous solution, the hydrophobic domains of a protein are generally buried in the interior of the molecule. To stabilize an emulsion, the hydrophobic domains of the protein should ideally be oriented toward the oil phase. The ease with which a protein is able to unfold (i. e., denature) to expose its hydrophobic domains, therefore, affects its emulsifying properties. The three-dimensional structure of proteins can be stabilized by both covalent and non-covalent interactions. Covalent interactions consist of disulfide bonds, both intra- and intermolecular. Several approaches have been used to modify disulfide bonds and to test whether the resulting protein has enhanced emulsifying properties [66, 67].

The improvement in functionality may be attributed to increased conformational mobility. Other attempts were the elimination of the cysteine residues using recombinant DNA technology [68,69], but the results not were conclusive. Molecules that contain crosslinks such as disulfide bonds are more rigid and less able to unfold. Such molecules are less effective in emulsion formation. The reduction of disulfide bonds enhances the emulsifying ability of some proteins as long as the molecules do not unfold to the point where there is a large increase in viscosity [70]. The content of disulfide bonds has been related to the emulsion capacity of complex mixtures of proteins such as whey protein concentrates. Small highly crosslinked protein molecules tend to perform poor emulsifiers. Protein flexibility is also affected by non-covalent interactions such as hydrogen bonding, van der Waal's forces, electrostatic links, and hydrophobic interactions [71].

Protein molecules may contain crosslinks of a non-covalent nature, e.g. salt bridges, or a covalent nature, e.g. disulfide bonds. These crosslinks lower the conformational entropy of the molecule which must be compensated by a decrease in binding energy. The presence of crosslinks adds greatly to the stability of the native protein structure and makes the molecules resistant to unfolding or denaturation. For example, simple unfolding is inhibited sterically by the presence of crosslinking because portions of the molecules are held in place by the crosslinks. Denaturation is also less likely because one of the driving forces of denaturation, an increment in conformational entropy, is greatly reduced [66-67]. In a non-crosslinked protein, if unfolding to a random coil structure can be induced, there is a very large gain in the number of conformations the molecule can assume. This gain in conformational entropy is a large driving force for the maintenance of the denatured state when the denaturing agents are removed. In contrast, a highly crosslinked protein cannot assume the same degree of random conformations and thus the increase in entropy is much smaller. This helps explain why molecules that contain large numbers of disulfide bonds are often resistant to denaturation.

Molecular conformation and aggregation. The conformation and aggregation of proteins depend on the relative magnitude of the various attractive and repulsive interactions which occur within and between molecules, as well as their configurational entropy [72].

It has shown the protein aggregation processes is an important factor in the emulsifying properties of proteins like wheat glutenins [73,74] and soy globulins [75,76]. Segments of one protein may be capable of forming strong hydrogen bonds with segments on another proteic molecule, which causes the molecules to aggregate. These junction zones usually involve hydrogen-bonded helical or sheet-like structures. Hydrogen-bonded junction zones tend to be stable, which stabilizes the oil globule covering web at low temperatures but dissociate as the temperature is raised above a certain value because the configurational entropy term dominates [59].

The role of secondary structure. Once a protein molecule reaches the surface it must be able to unfold enough to expose hydrophobic groups if it is to function as an emulsifier. At this point, it is important to highlight the relevance of the secondary structure content [70,77] and the flexibility of the protein [65]. It is widely recognized that the interaction of the amino acid side chains with water is a major factor in determin-

ing the native structure of proteins [78,81]. The side chains of hydrophilic residues seek contact with water, whereas the side chains of hydrophobic residues avoid this contact. Most a-helixes in proteins consist of both hydrophobic and hydrophilic residues, except for proteins with transmembrane a-helixes, whose residues are basically hydrophobic [82-84]. The amphipatic a-helix is a structural feature which has previously been proposed as favouring good emulsifying properties [85] and which contributes to their surface activity[71,77,86]. This motif is also common to many proteins in physiological systems where binding to an interface or a non-polar ligand is involved (e. g., apolipoproteins [87]. Furthermore, *in vitro* studies have shown that the presence of an interface can induce or increase the degree of a-helix formation [88]. The average hydrophobicity and hydrophobic moment over 60 helixes have been compared by Eisenberg *et al.* [78] who found that helixes could be classified into three groups according to their helix parameters. Each group represented an *in vivo* location: (1) globular proteins; (2) transmembrane helixes; and (3) helixes which were believed to seek the surface between aqueous and non-aqueous phases ("surface-seeking helices"). The surface-seeking helixes form a subset of globular helixes, which is not surprising, given the diversity of structures within globular proteins. **Fig. 2** is based on the data from Eisenberg *et al.* [70,78] and data from additional peptides [70].

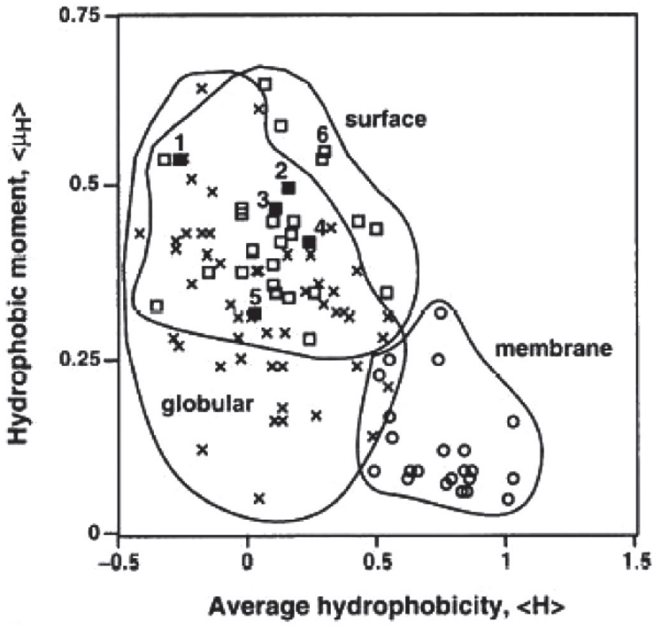

Fig. 2 Hydrophobic Moment Plot of a-Helices showing the separation between Globular, Membrane and Surface-seeking/Emulsifier Helices. Globular helices (x) are those derived from globular proteins; membrane helices (?) are those which have tendency to align at the interface between polar and non-polar phases. Emulsifier helices (¦) are those from molecules which have good emulsifying activity. The *y*-axis is the average hydrophobic moment per residue ‹m_H›, and the *x*-axis is the average hydrophobicity per residue ‹H›. Where segments are greater than 18 residues, two points have benn plotted, one representing the average value ant the other representing the 18-residue segment (theoretically five complete turns of an a-helix) having the highest Hydrophobic moment [70].

c) Characterization of protein emulsifying properties

The determination of meaningful emulsion data with complex food products is difficult. Much of the experimental work with model systems has been done in very dilute solutions. The surface pressure or interfacial tension is often the quantity measured. With a food product the relevant information is concerned with the question: How much lipid can be emulsified and how long will it be stable to coalescence and / or creaming? The situation in food products is also complicated by the presence of other surface active molecules in addition to the proteins present [89-92].

A variety of tests has been applied to indicate the value of a protein in an emulsion. A range of experimentally controllable parameters will alter the measured emulsification properties of a protein being evaluated. These include: type of equipment used to produce the emulsion, energy input into the system, amount o protein used, phase volumes used, ionic strength, pH and type of oil used. In **Table 3** some of the properties and tests used in the evaluation of the protein emulsifying properties are shown.

d) Emulsifying properties improvement

Proteins are widely utilized as food emulsifiers However, in recent years, food materials with new functionality have become strongly desired by the increasing variety of demands. Much attention has been paid to the preparation of proteins with new functions, and in particular, protein modification has captured wide interest. The modification of a protein usually refers to physical, chemical or enzymatic treatment which change its conformation and structure and consequently its physicochemical and functional properties [93-94]. To improve the functional properties, and particularly, the emulsifying properties of food proteins, various chemical and enzymatic modifications have been carried out such as alkylation, glycosilation [95,96], esterification [97], phosphorylation [94], amidation, deamidation [98,101], lipophilization [102], hydrolysis [103], disulfide-mediated polymerization [104], covalent attachment of gluconic acid, cross-linking with transglutaminase, and conjugation with polymers [105] and Polyethylene Glycol [106] or Carboxymethyl cyclodextrins [107]. In particular, multiple improvements to protein functions can be expected by conjugating with a charged polymer due to the difference in molecular weight or charge of the chemical species conjugated [108-110]. However, chemical modifications of proteins generally suffer from a lack of control in the extent of derivatization attainable, often yielding polydisperse subproducts. Recent advances in recombinant deoxyribonucleic acid technology offer the opportunity to relate systematically

Table 3. Tests utilized in the evaluation of proteins emulsifying properties[a]

Property	Test
Emulsifier Efficiency	Emulsifying Capacity Emulsion Stability Index Interfacial Tension Interfacial Rheology
Microestructure and Droplet Size Distribution	Microscopy Static Light Scattering Dynamic Light Scattering Electrical Pulse Counting Sedimentation Techniques Ultrasonic Spectrometry NMR Neutron Scattering Dielectric Spectroscopy Electroacoustics
Dispersed-Phase Volume Fraction	Proximate Analysis Density Measurement Electrical Conductivity Alternative Techniques
Droplet Crystallinity	Dilatometry NMR Thermal Analysis Ultrasonics
Droplet Charge	Electrophoresis Zetasizer© Electroacoustics

[a] From McClements, D. J.[57]

well-defined alterations in the primary sequence to changes in protein functionality. Using oligonucleotide-directed mutagenesis, one can now use a synthetic sequence of proteins. Incorporation of the altered genes into an appropriate host can lead to the production of the modified protein for structure-function relationship studies. Site directed mutagenesis can also be achieved by using PCR**.** The recombinant deoxyribonucleic acid techniques may eventually provide the means to engineer proteins and enzymes with improved functional properties [111,112].

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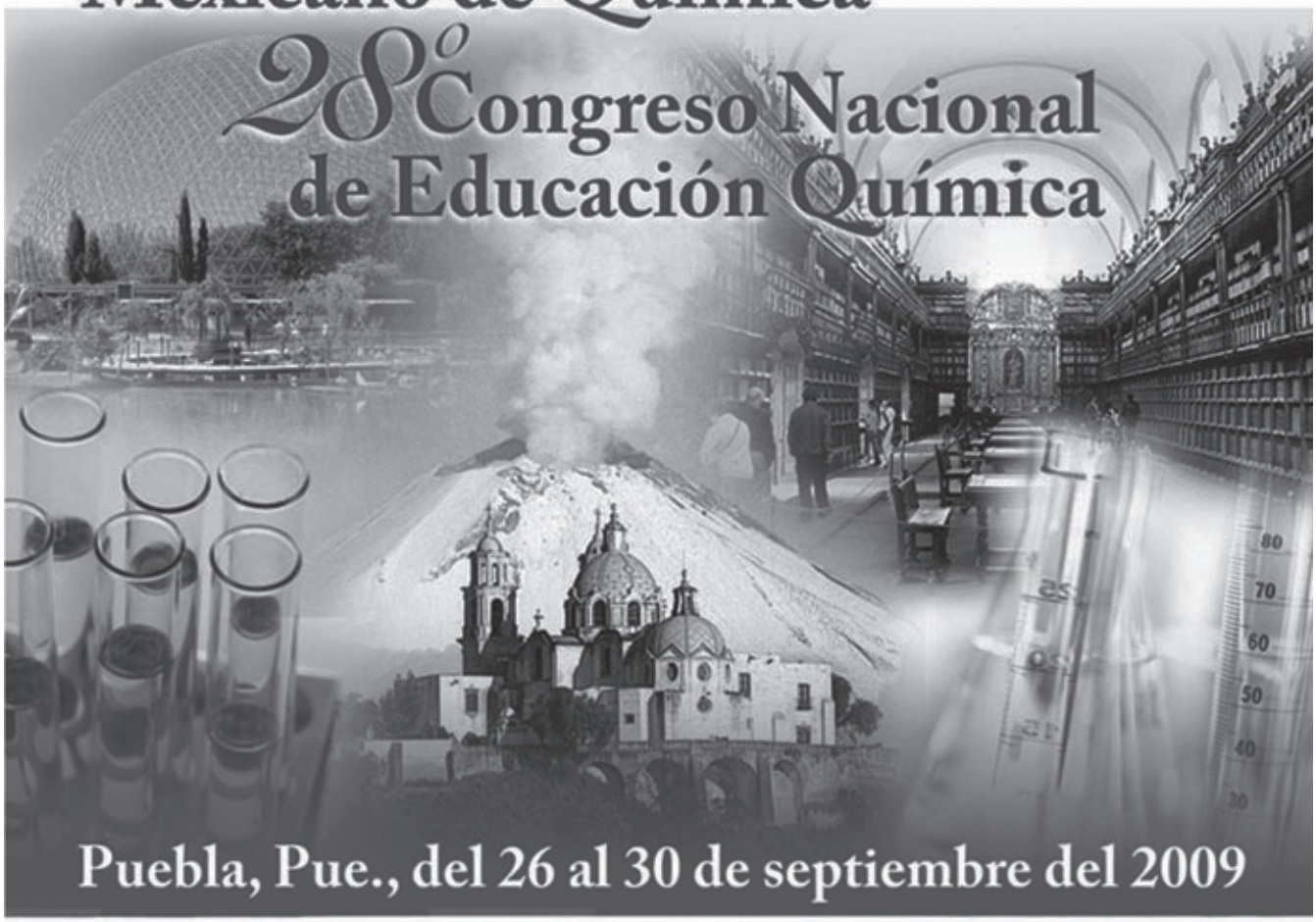

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