The role vegetable proteins to stabilize emulsion: a mini review

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Abstract. The critical aspect in forming emulsion is the selection of the right emulsifier. Emulsifiers are facilitating emulsion formation and promoting emulsion stability. Protein is the emulsifier that is often found in the food industry, because it has an active surface containing a mixture of hydrophilic and hydrophobic amino acids along the polypeptide chains. Protein have emulsification properties due to their amphipathic properties (having hydrophobic and hydrophilic groups) are the properties of proteins as emulsification because these proteins are able to form a layer at the oil-water interface. However, protein-stabilized emulsions are sensitive to charge changes, and proteins tend to diffuse slowly towards the interface compared to emulsifiers with smaller molecular weight. The purpose of this literature review is to determine the ability of vegetable proteins to stabilize emulsions.

1. Introduction

According to the Indonesian Pharmacopoeia, an emulsion is a preparation containing a liquid medicine material or drug solution, dispersed in a liquid carrier, stabilized with a suitable emulsifying agent or surfactant. Meanwhile, according to the National Formulary, an emulsion is a preparation in the form of a mixture consisting of two liquid phases in a liquid phase dispersion system, one of which is very finely and evenly dispersed in the other liquid phase, generally stabilized by an emulsifying agent. Special types of colloidal dispersions, such as emulsions, have one dimension between 1 to 1000 nm. The internal phase is commonly known as the dispersed phase, while the external phase is commonly mentioned as the continuous phase. The type of colloidal system formed by the emulsion is rather special because the colloid droplets often exceed the limited size of 1000 nm [1]. Emulsion can be used as a final product or during product processing in various fields including the food industry, agricultural industry, pharmaceuticals, cosmetics, and in food form.

In an emulsion, one of the liquid phases is usually polar while the other is relatively non-polar. Determination of the type of emulsion depends on a number of factors. If the phase volume ratio is very small, then the dispersed phase is often the phase that has a smaller volume [2]. Emulsion is divided into two types: (1) Oil in water (o/w): the oil phase is dispersed as droplets in the entire outer phase of water and (2) Water in oil (w/o): the water phase is dispersed as droplets in the oil outer phase [3].
There are three methods to make emulsions on a small scale, namely dry gum method, wet gum method and Forbes bottle method [4]. The dry gum method is also known as the 4:2:1 method. The emulsifying agent used in this method is in the form of a dry gum. The emulsion base is prepared with 4 parts (volume) of oil, 2 parts of water, and 1 part of gum added in the preparation of the emulsion base. In this method, water is added to the oil before the emulsifying agent. Wet gum method has the same proportions of oil, water, and gum that is used in the dry gum method, but the mixing order is different. Emulsifying agent is added to water (which can be dissolved) to form mucus, then slowly the oil will combine to form an emulsion. Finally, Forbes bottle method is used for volatile or less viscous oils.

2. Protein as an emulsifier

Protein is one of the emulsifiers that often found in the food industry, because it has an active surface that contains a mixture of hydrophilic and hydrophobic amino acids along the polypeptide chain. Proteins have emulsification properties due to their amphipathic properties (having hydrophobic and hydrophilic groups) and the ability to form a layer on the water oil interface [5]. However, protein-stabilized emulsions are sensitive to changes in charge, and proteins tend to diffuse more slowly toward the interface than emulsifiers with smaller molecular weights. Proteins tend to aggregate at the isoelectric point, so protein-stabilized emulsions need to have a pH that is further from the isoelectric point to prevent aggregation [6]. Therefore, based on these problems, protein-stabilized emulsions can limit the processing and production of beverage industry.

Phospholipids are one of the ionic emulsifiers and include low molecular weight emulsifiers that are used to reduce surface tension [7]. Low molecular weight (phospholipid) emulsifiers are more effective at reducing the surface tension of emulsions than high molecular weight (protein) emulsifiers. However, low molecular weight emulsifiers are less effective against coalescence. This is because the steric repulsive force among the phospholipids droplets is very effective in fighting aggregation. The phospholipids droplet is covered with protein. When proteins form complexes with phospholipids, there is an increase in emulsion stability even though the emulsion pH is around the isoelectric point [8]. In addition, the addition of phospholipids could increase the stability of emulsions containing native or denatured proteins [9]. Hydrophobic interactions between denatured proteins and phospholipids play an important role in the formation of protein-phospholipid complexes.

The mechanism for the formation of protein-phospholipid complexes begins with ionic or electrostatic attraction, which is then followed by hydrophobic interactions to stabilize the complex. The electrostatic bond of attraction occurs because of the opposite charge between the protein and the phospholipid. Positively charged molecules of phospholipids (example: choline) will attract each other with negatively charged molecules of protein (example: aspartyl, glutamyl), or negatively charged phosphate groups of phospholipids and positively charged of proteins (example: lysyl or guanidyl, amyl) [10]. The presence of protein adsorption on the hydrophobic part of the phospholipid tail group can increase the density of the layer surrounding the oil droplets, so it can reduce the risk of oil droplets sticking together [11]. Both stages of the mechanism are influenced by environmental factors of the emulsion, namely pH and heating temperature. Electrostatic bond is influenced by the pH factor of the emulsion, while hydrophobic bond is influenced by treatments to denature proteins such as heating temperature, so it is important to know the effect of pH and heating temperature on the mechanism of these two bonds in protein-phospholipid complexes.

3. Functional properties of proteins

The use of protein in a food should fulfill some of the expected characteristics and be known as various functional properties. Functional properties of proteins are physicochemical properties of proteins that affect the role of proteins in the food system during preparation, processing, storage and consumption, as well as their contribution to the quality and sensory properties of the food system. During food processing the combination of several physical and chemical properties which are functional properties of proteins cannot be measured using a single physical and chemical test [12].
Functional protein properties can be divided into three main groups, namely: (1) hydration properties, such as water retention or solubility; (2) surface properties, such as shaping and emulsification; and (3) interactions between proteins, such as gelation[13]. Various functional properties of proteins affect the role of proteins such as: solubility, water absorption, oil absorption, emulsion capacity and stability, as well as foam capacity and stability, coagulation, and gelation (gel formation). The functional properties of these proteins are closely related to the type of protein itself. The type of protein in question is related to these protein sources, such as meat protein, milk protein, soy protein, and so on. Aspects and relationships among functional properties of proteins in food systems can be seen in Figure 1.

The functional properties of proteins are influenced by the molecular properties of proteins that can be modified by processing treatment, environmental factors, and interactions with other components, such as carbohydrates, lipids, and other proteins. Environmental conditions such as pH, ionic strength, type of salt, water content, oxidation-reduction potential, can affect the functional properties of protein in a food. The processing treatments are heating, drying, pressing, and freezing. During storage, the functional properties of proteins generally undergo changes such as protein aggregation, denaturation, enzyme activity, lipid oxidation, and damage by ice crystals [13].

Figure 1. Aspects and relationships among protein functional properties in food systems [13].

Functional characteristics of protein are protein properties that can affect the final product. In addition, the most important functional characteristic of protein is protein solubility, because protein solubility can affect other characteristics including water absorption, oil absorption, gel capacity, emulsion stability and foam formation capacity.

3.1. Solubility
The functional properties of proteins are generally influenced by protein solubility. Insoluble protein has limited use in food. Protein solubility is a balance between interactions among proteins with the interactions between proteins and solvents. The main interactions that affect solubility are hydrophobic and ionic interactions. Hydrophobic interactions are interactions among proteins that reduce solubility, while ionic interactions are interactions between proteins and water, thereby increasing solubility. The mechanism of the process can occur in 3 stages. The first stage is in the form of changing the solute molecules from the pure solute phase to the vapor phase. The second stage is the formation of holes in
the solvent for the joining of the solute molecules. The third stage is the stage when the free solute molecule phase fills the holes in the solvent [13].

![Figure 2. Hypothesis of the dissolving process stages.](image)

After being tested in liquefaction process in which hydrolyzate is dissolved in the water, it is proven that the duration of hydrolysis depends on the solubility of the protein. Significantly solubility is an important factor related to temperature. Perfect solubility can be considered in the optimum temperature in the hydrolysis process [14]. The protein fraction based on its solubility is divided into 4 groups, namely albumin, globulin, glutelin and prolamin. The distribution of proteins based on their solubility is included so that it is known which protein fraction has the most prominent functional characteristics. Protein fractions that have high solubility will increase the emulsion capacity and foam formation capacity.

a) Albumin dissolves in water, but dissolves in salt solutions and is insoluble in high concentrations of salt solutions as well as can coagulate by heat.

b) Globulins, proteins that are insoluble in water, dissolve in dilute salt solutions, precipitate in high concentrations of salt (salting out), and are coagulated by heat. Examples are myosinogen in muscle, albumin in egg yolk, and legumes in beans.

c) Prolamin (Gliadin), which is a protein that dissolves in 70-80% ethanol, but is not soluble in water, salt solution or absolute/pure ethanol. Examples are gliadin in wheat, zein in corn, and hordaine in barley.

d) Glutelin, which is a protein that is not soluble in neutral solvents, salt solutions or ethanol, but can dissolve in alkaline solutions or dilute acid. Examples are glutelin in wheat and orizenin in rice.

Protein solubility is the main protein characteristic that can be selected for use in liquid food and beverage products. Proteins with high solubility mean that protein molecules can spread well [15]. Protein solubility is influenced by several internal factors, namely protein molecular weight, amino acid composition and protein conformation. External factors that affect protein solubility are pH, temperature, type of solvent, ionic strength or salt concentration [16]. Proteins that have high solubility, protein applications can be carried out widely compared to proteins with low solubility. Proteins derived from different materials will produce different solubility so that they have their respective characteristics which are influenced by pH, salt concentration and temperature factors. Salt concentration and pH adjustment are easier to do to prepare protein before being applied in the beverage industry than temperature adjustment [16].

3.2. Water absorption

Water absorption is defined as the ability to hold water due to force, pressure, centrifugation and heating. This parameter is commonly used as a determining and limiting factor in the use of protein in food. The
mechanism of the water-binding process by proteins occurs in several stages. Ionic groups with high affinity will experience first under conditions of low water activity, followed by polar and nonpolar groups. Free water molecules have higher affinity than permanently bound water molecules. Water that is not directly attached to the surface of the protein but remains in the hydration layer is known as free water. The exchange between free and bonded water occurs simultaneously and constantly. The interaction among water molecules and between water molecules with amino acid side chains in the polypeptide chain occurs in the form of hydrogen bonds. The hydrogen bond of water molecules occurs as a result of the partial charge difference from the difference in electronegativity between oxygen and hydrogen [15].

Water absorption is influenced by protein concentration, pH, ionic strength, temperature, other components in food such as fats, salts, hydrophilic polysaccharides, heating and storage conditions. Protein will bind more water when the pH is above or below the pH. This condition is related to the increase in charge and repulsive force. The binding capacity of protein water is generally greater at pH 9-10 than another pH. Water binding capacity is one of the protein characteristics chosen for use in the food industry, especially in puddings and yogurt because it produces a thick texture and prevents separation [7]. Changes in protein conformation during processing occur generally due to heating. Heating causes the opening of the polypeptide chain structure and is known as denaturation. The water-binding capacity of denatured proteins generally increases 10% greater than native proteins. Denaturation which causes protein aggregation will decrease the binding capacity of water due to the interaction among these proteins.

3.3. Gel capacity
The gel formation process occurs in 2 stages, namely the protein denaturation process is the initial stage of gelation caused by heating and protein aggregation is the second stage where the formation of a three-dimensional structure occurs. The denaturation process changes the secondary, tertiary and quaternary structure of proteins without breaking the covalent bonds [7]. This causes reactive groups of the same or adjacent proteins to open and form bonds. The bonds among the reactive groups of the protein will hold the liquid to form a gel. Type of bonds that can affect the formation of network structures during the gelation process are hydrogen bonds, salt bonds, hydrophobic bonds and disulfide bonds. The occurrence of hydrophobic interactions is caused by increasing temperature and unstable hydrogen bonds. Gel formation is influenced by several factors, namely environmental factors (ionic strength and pH), protein preparation method, processing conditions, heating and cooling time. The capacity of food protein gel affects the texture and juiciness of the food product. Proteins that have the ability to form gels can be applied to the edible film industry, jelly and tofu products [7].

3.4. Oil absorption
Oil absorption is a property that can indicate the interaction of a material with oil [17]. Oil absorption is defined as the physical binding property of fat/oil by protein. The ability of proteins to absorb, hold and react with fats in emulsions and food systems is required in food formulations [11]. Oil absorption is one of the properties possessed by protein and it is very necessary to assist the use of this protein in food processing in the form of an emulsion. Source of protein, processing conditions, the composition of additives, particle size, and temperature are factors that affect protein binding to oil. The absorption of oil is determined by the binding of the oil by the nonpolar portion of the protein. The capacity of proteins to hold lipids is influenced by the protein-lipid interactions and the spacial arrangement in the lipid phase which is determined by the interactions among these lipids. The interactions between proteins and lipids are influenced by hydrophobic, electrostatic, hydrogen, and non-covalent bonds. The hydrophobic bond is a very important bond in stabilizing the protein-lipid complex.

4. Emulsion capacity and stability
An emulsion is a dispersion system of one or more liquids which are immiscible. The mixture is stabilized by an emulsifying agent, which can form a film layer that connects among the liquids. Proteins
can adopt oils because of the presence of residues in hydrophobic amino acids that can be detached from the hydrogen bridge matrix around the water molecules. This results in the replacement of water molecules contained in the hydrophobic part of the barrier layer between oil and water. Emulsions in food can be of 2 types, namely oil in water and water in oil [11].

![Protein Structure](image)

**Figure 3.** Illustration of emulsification by protein.

Emulsion capacity is the amount of oil (ml) that can emulsify 1 g of protein under certain conditions. Meanwhile, emulsion stability is the ability of the emulsion to maintain its dispersion without separation. Emulsion capacity and stability are influenced by protein origin and concentration, pH, ionic strength, and system viscosity. Proteins that are suitable for use as emulsifying agents should have low molecular weight, balanced amino acid composition between charged residues, polar and nonpolar, good solubility in water, as well as can form a good hydrophobic surface and stable conformation [11]. Comparison of the amount of hydrophilic-lipophilic amino acids of proteins capable of being adsorbed at the water-oil interface with a lipophilic mechanism that binds to the oil side and hydrophilic binds to the liquid phase. Utilization of emulsion capacity is used in food products such as cakes [7].

5. **Emulsion stabilization mechanism**

According to the laws of thermodynamics, the emulsion system is unstable, because the system will tend to move to the lowest energy level. Normally, water and oil will separate to form two stable phases. Emulsions can be stabilized by decreasing the surface tension of the oil droplets or by increasing the density of the layer surrounding the oil droplets [18]. The stability of the emulsion depends on the interaction between various attractive and repulsive forces between the droplets which are influenced by electrostatic and steric stabilization.

The attractive electrostatic stabilization (Figure 3) occurs due to the difference in charge between the surface of the oil droplet and the ionic emulsifier. The same type of emulsifier will be stabilized by all the droplets in the emulsion so that they have the same charge. Electrostatic interactions play a major role in preventing droplets from approaching each other for aggregation. Therefore repulsion is an electrostatic interaction between droplets having the same charge. The high electrostatic force among the oil droplets causes the higher stability of the emulsion. Therefore, the surface charge of the droplet is highly dependent on the interaction between the two droplets. Because they can stabilize emulsions electrostatically, proteins and phospholipids are also called ionic emulsifiers [19].

Meanwhile, steric stabilization (Figure 4) is stabilization of oil droplets by non-ionic macromolecules by forming a physical barrier, to prevent contact among oil droplets. High molecular weight polymers such as proteins can be adsorbed on the surface of the dispersed phase droplets, thus providing a barrier to physical contact. Proteins have a side chain of hydrophobic and hydrophilic polypeptides, where the
hydrophobic side will be located in the oil phase, while the hydrophilic side is on the water side in the interface. When the particles that have been covered by the polymer come close to each other, they create a repulsive force that separates the particles from each other. Proteins can stabilize steric by forming a thick layer at the oil-water interface [9].

![Figure 4. Emulsion stabilization mechanism: (a) the difference in charge provided by ionic emulsifiers helps to stabilize the emulsion, while steric stabilization (b) will physically prevent oil droplets from forming aggregates [20].](image)

Proteins have an active surface because they contain a mixture of hydrophilic and hydrophobic amino acids along their polypeptide chains. Therefore, proteins have emulsification properties due to their amphipathic properties (having hydrophobic and hydrophilic groups) and the ability to form a layer on the surface of oil droplets. Two protein mechanisms in preventing oil droplet aggregation are steric and electrostatic stabilization [20]. The repulsive electrostatic stabilization can be produced because the protein contains amino acids with side chains that have a negative (-COO-) or positive (-NH3+) charge. The formation of a charge layer surrounding the oil droplets causes the droplets to repel each other to prevent blow. Meanwhile, steric repulsion stabilization occurs by forming a thick layer on the oil droplets. Because protein has high molecular weight, protein can form a thick physical barrier layer on the oil droplet surface to prevent the aggregation of oil droplets [21]. The hydrophobic amino acids contained in the protein core must come out and be adsorbed on the surface of the oil droplet. Meanwhile, hydrophilic amino acids in the aqueous phase act as a steric barrier against coalescence and flocculation [22].

Unlike emulsifiers with small molecular weights which can diffuse rapidly to the interface to form a good emulsion, proteins tend to take up a large space and have a slow diffusion rate [19]. The hydrophobic surface affects the ability of the protein to adsorb oil at the interface, where the greater the hydrophobic properties of the protein, the higher the emulsion capacity. Meanwhile, the surface of the protein charge affects the rate of diffusion of the protein towards the oil droplet surface [23]. Proteins can rearrange their structure on the oil droplet surface. The hydrophobic part of the protein will open in the lipid phase and the polar (hydrophilic) part will open to the water phase. The hydrophobic part will be inside the molecule, while the hydrophilic part will be on the surface of the molecule. The closed globular shape and the large molecular size of the protein cause the emulsification properties to be limited [21]. The temperature changes play a role in the protein denaturation process. The ability of proteins to diffuse, surface charge, ease of unfolding (denaturation), and forming a layer on droplets will affect the surface tension [24]. A study by [25] on the protein adsorption kinetic in the interface stated that protein is a good emulsifier if it has the following properties: (1) high diffusion and adsorption rate towards the interface, (2) fast unfolding capability and interface re-orientation, and (3) intra-droplet interaction on the interface. Sources of vegetable protein used to stabilize the emulsion are:

5.1. Soybean

Soybean oil contains unsaturated fatty acids such as -linolenic acid (omega 3), linoleic acid, -linolenic acid and arachidonic acid (omega 6) and oleic acid (omega 9). Soybean oil also contains lecithin which can help egg yolks to form a good emulsion [26]. Lecithin has hydrophobic and hydrophilic groups that...
are able to unite oil and water to produce an emulsion system. In foods, lecithin provides about a dozen functions, including as an emulsifier, as a wetting agent, for viscosity reduction, as a release agent, and for crystallization control. The widely recognized properties of lecithin are it can be used as an emulsifier and it also has other functional properties which make it commonly incorporated in a variety of foods including butter, margarine, chocolate, macaroni and noodles, baked goods, sweets, instant cocoa drinks, ice cream, milk powder, and as a crystal inhibitor and antioxidants in fats and oils [27]. Vegetable lecithin contains phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), obtained commercially from oil-containing seeds such as soybeans, sunflower seeds, and horseradish seeds. Lecithin with surface active properties is used as an emulsifier in a variety of food, feed, pharmaceutical and technical applications [28].

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Proteins contained in coconut milk (globulins, albumin) act as natural emulsifiers, but their emulsification power decreases along with the decrease in protein quality in storage [29]. Pure coconut milk contains 54% water, 35% fat and 11% non-fat solids. The emulsification ability of internal protein because coconut milk protein can interact and the concentration of coconut milk fat globules so that it can lead to separation in phases [30].

Lecithin has a structure like fat but contains phosphoric acid. Lecithin has a polar and non-polar structure. The polar group contained in the phosphate ester is hydrophilic and has a tendency to dissolve in water, while the nonpolar group contained in the fatty acid ester is lipophilic which tends to dissolve in fat or oil.

5.2. Plant protein (cereals)

Cereals are inexpensive source of vegetable proteins that easy to find in our daily life, which is important both as nutritional source and in techno-functional point of view. Cereal proteins are currently utilized to stabilize PE, but it has poor solubility in aqueous medium hence limiting its application to many food products. Therefore, physical, chemical or enzymatic modification of cereal proteins to increase solubility and other functional properties has been widely studied [31].

In plant seeds there is a type of protein, namely prolamin (for example in corn and wheat), which has excellent water and heat resistance. To stabilize the ether solvent, prolamin particles are also used frequently. Water-insoluble proteins are usually used to stabilize O/W (oil in water) emulsions where water is a continuous phase, this is due to the high surface hydrophobicity. [32] It is investigated the ability of zein protein particles to stabilize emulsions in which the oil was a continuous phase (W/O) and reported that the resulting emulsion was unstable and also exhibited a reversible phase inversion. Thus, to help stabilize the ether solvent, researchers usually add particles or other reagents such as lecithin [8]. Compared to the production of animal protein, the cost of producing vegetable protein is much cheaper and sustainable [33]. In addition, good emulsifying and emulsifying properties were obtained from unconventional plant proteins. Thus, in the food industry in the future, vegetable protein is expected to be more widely used.

5.3. Sunflower seeds

Vegetable oils, such as canola oil, peanut oil, sunflower seed oil and olive oil, are commonly used in making mayonnaise. The use of vegetable oil acts as an internal phase that will affect the viscosity of the mayonnaise [34]. Each type of vegetable oil has different fatty acids. Sunflower seed oil is a vegetable oil that contains high unsaturated fatty acids, which are about 90% linoleic acid and linolenic acid. The composition of sunflower seed oil ranges from 23-45%. The linoleic acid and oleic acid of sunflower seed oil contain 44-72% and 11.7% respectively. Sunflower seed oil is used for various purposes such as cooking oil, raw material for cosmetics, making margarine and medicine, in addition, 13-20% of protein from oil cake or dregs can be used as animal feed. Sunflower seeds are better for health than corn oil, peanut oil and soybean oil because sunflower seeds are classified as low cholesterol oils so they are very safe for consumption.
5.4. Coconut

Virgin Coconut Oil (VCO) is used as the dispersed part in the emulsion system in the manufacture of pasta. VCO is an oil that comes from processing fresh coconut flesh. The manufacturing process does not use too high heating and does not use additional chemicals. VCO processing does not go through the stages of purification, bleaching, and deodorization. VCO is one of the food sources of fat that is currently in great demand by people because of its health benefits. Compared to other vegetable oils such as palm oil, soybean oil, corn oil, and sunflower seed oil, VCO has several advantages, namely high lauric acid content. Lauric acid in the body will be converted into monolaurin, a monoglyceride compound that has antiviral, antibacterial and antiprotozoal properties, so that it can increase the human body's resistance to disease and accelerate the healing process. In addition to lauric acid and antioxidants, VCO has a distinctive coconut aroma, is clear, colorless, not easily rancid, and lasts up to two years [35]. Proteins contained in coconut milk (globulins, albumin) act as natural emulsifiers, but their emulsification power decreases along with the decline in protein quality during storage. Pure coconut milk contains 54% water, 35% fat and 11% non-fat solids. The emulsification ability of protein in coconut milk is due to the protein in coconut milk being able to interact and envelop fat globules so that it can inhibit phase separation [30].

5.5. Gum arabic

In the metastability of oil-in-water emulsions used in the agro-food industry, gum arabic is usually used which is a natural hydrocolloid that also has heterogeneous characteristic. Due to its binding and stabilizing properties such as oil-in-water emulsions, particularly in flavored drinks, Gum Arab which is an exudate derived from the acacia tree is commonly found in sub-Saharan countries [36]. Sap product (resin) produced from tapping the sap on the stems of legumes (legumes. Gum arabic is widely used in the food and chemical industries as a beverage mixture to reduce water surface tension and stabilizer. Gum arabic contains protein that plays an important role in maintaining its emulsification ability but is easily denatured. All gum arabic molecules are composed of the same sugars, namely galactose, arabinose, rhamnose, and glucuronic acid which are neutralized by calcium, potassium, sodium, and magnesium salts [29]. Gum arabic can increase the viscosity of the dispersing phase so that it can act as a stabilizer.

6. Conclusions

Vegetable proteins can be used as stabilizers and emulsifiers when applied to food products which are not much different from emulsions made from animal protein. Amphipathic properties (having hydrophobic and hydrophilic groups) are the properties of proteins as emulsification because these proteins are able to form a layer at the oil-water interface.

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