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Structural and biochemical factors affecting the digestion of protein-stabilized emulsions

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ABSTRACT

During the last two decades, important insights into the physico-chemical properties of oil-in-water emulsions under various food-processing conditions have been gained. Much of this research has focused on understanding how the various types of proteins, in particular milk proteins, function as emulsifiers and stabilizers in food emulsions. Recently, attention has been given to understanding the behavior of emulsions during gastrointestinal digestion, using mainly in vitro models. These studies have provided useful information on how various types of emulsions behave in a wide range of physical (e.g. shear, temperature) and biochemical (e.g. dilution, pH, pepsin, pancreatin, mucins, bile salts) environments that are relevant to digestion. This knowledge may allow manipulation of the physico-chemical and interfacial properties to modulate lipid ingestion and to improve the bioavailability of lipid-soluble nutrients. However, many of these findings will need to be validated in in vivo models and human clinical trials. Limited studies in humans have shown that the extent and the duration of postprandial lipemia are positively related to the pathogenesis and progression of coronary heart disease. The rate of digestion of lipids also appears to be important in satiation and subsequent energy regulation.

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1. Introduction

Lipids are an essential component of the human diet, as they are a dense source of energy and a carrier of lipid-soluble micronutrients and bioactive molecules. Lipids also give specific textural and sensorial characteristics to foods. Overconsumption of energy-dense, high fat foods is considered to be a major contributor to obesity and cardiovascular diseases. There are two main solutions to managing fat intake: reducing the amount of lipids in processed foods at the cost of taste and flavor or structuring lipids to reduce their digestibility and bioavailability. There has been considerable research into establishing a relationship between food structure and sensory properties, particularly in connection with the role of lipids in foods [1]. This research has enabled the food industry to develop low fat food products with acceptable quality. The route to developing food products with low lipid digestibility is complicated and requires an understanding of how lipids are processed in the gastrointestinal tract and their physiological responses.

Lipids occur naturally in foods such as meat, plants, nuts, seeds and milk. Plant, nut and seed lipids are stored in oil bodies, which are oil droplets that are surrounded by a monolayer of phospholipids and embedded specific proteins called oleosins. All mammalian milk lipids have the same common structure, i.e. a fat globule that has a triglyceride core surrounded by a milk fat globule membrane, which consists of phospholipids, glycoproteins and cholesterol. In most processed foods, lipids, from plant or animal sources, are stabilized in the form of emulsions. The most widely consumed examples are dairy products, chocolate, sauces, mayonnaise, soups, spreads and dressings. Proteins, phospholipids, monoglycerides and diglycerides are also often used as emulsifiers and stabilizers in food emulsions. The digestion and absorption characteristics of lipid emulsions can be controlled by choosing certain critical parameters, such as the size of the lipid droplets, the type of emulsifier, the type of emulsion and the type and structure of the triglycerides [2••,3••,4•].

The development of protein-stabilized emulsions relies on understanding the interfacial behavior of proteins, the interactions of emulsion droplets and the influence of environmental stresses (various solution and processing conditions) on the functionality of the emulsion. Recently, there has been a great deal of research into developing a better understanding of the behavior of protein-stabilized emulsions during in vitro gastrointestinal digestion [2••,3••,4•,5••,6•,7••]. However, most of this research has been carried out using relatively simple, well-defined systems and in vitro models have been used to follow digestion processes. The major emphasis has been on developing a fundamental understanding of the influence of gastrointestinal conditions (e.g. pH, ionic strength, enzyme action, bile salts, mucins) on the stability of emulsions and the digestibility of proteins and lipids. In this review, we focus primarily on the factors that affect the digestibility of protein-based lipid emulsions and discuss how the structure of an emulsion influences the various steps involved in the digestibility of lipids.

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2. Proteins in oil-in-water emulsions

Many proteins exhibit excellent surface activity, which permits their use as food ingredients in the preparation of emulsions and foams [8,9*,10**]. Their surface activity can be attributed to the molecular flexibility and amphiphilicity of their polypeptide chains. The molecular flexibility, i.e. the ability of a protein to undergo rapid conformational change when it is transferred from one environment to another, is dependent on the way in which the polypeptide chain is folded in three-dimensional space, i.e. its conformation [11]. Proteins that are highly compact and ordered possess poorer surface activity than proteins that have disordered, open structures. Rapid conformational change at an interface is essential for the protein to reorient its hydrophobic amino acid residues in the oil phase and its hydrophilic residues towards the aqueous phase. By adsorbing at the interface, the protein reduces the free energy of the system and hence the interfacial tension. The nature of the distribution of hydrophilic and hydrophobic residues and charged amino acid residues on the surface of a protein is also important in determining its surface activity. Surface hydrophobicity, which relates to the number of hydrophobic patches on the surface of a protein, is considered to play an important role in the initial anchoring and retention of a protein at the oil–water interface [8].

Oil-in-water emulsions are composed of oil droplets, with average diameters ranging from 0.5 to 5 μm, that are enveloped by a continuous film of surfactant material that stabilizes the droplets. The formation of emulsions requires intense shearing of oil and water mixtures in the presence of emulsifiers (e.g. proteins), using colloid mills, high speed blenders and high pressure valve homogenizers. The intense shear flow and turbulence cause dispersion of the oil phase into small oil droplets. Simultaneously, the surface-active proteins become adsorbed at the oil–water interface, lowering the surface tension and thus facilitating further droplet disruption [12]. The adsorbed proteins form a stabilizing interfacial layer at the oil droplet surface, which retards coalescence. Once the proteins are adsorbed, their conformation may change considerably, as part of the polypeptide comes into close contact with the hydrophobic oil surface. Proteins with relatively disordered, open structures (such as casein) undergo relatively rapid conformational changes upon adsorption, whereas more rigid globular proteins (such as serum albumin) exhibit slow conformational changes. Such conformational changes can be seen as a form of interfacial denaturation of the protein. The amount of protein adsorbed at the interface per unit surface of oil phase is defined as the surface protein load (mg/m^2), which indicates the amount of protein required to make an emulsion with a desired oil volume and droplet size. The surface protein load is affected by protein type, protein concentration, energy input, state of protein aggregation, pH, ionic strength and temperature [12,13].

The most commonly used sources of proteins in preparing food emulsions are derived from milk, soybean and eggs. The proteins derived from milk have been extensively studied for their formation and stabilization of emulsions as they exhibit good surface-active properties and form interfacial layers with desirable rheological properties. Milk proteins fall into two categories: whey proteins, which are typical globular proteins; and caseins, which have rather flexible structures and lack regular secondary and tertiary structures. The role of caseins and whey proteins in stabilizing oil-in-water emulsions has been thoroughly investigated and published [10**,11–20]. In emulsions formed with sodium caseinate or whey proteins, the surface protein load increases with an increase in protein concentration until it reaches a plateau value of about 2.0–3.0 mg/m^2 [18]. Whey proteins (such as β-lactoglobulin) undergo partial unfolding and reorganization of the native protein conformation at the interface and form a compact adsorbed layer that is about 2 nm thick [18,19]. This partial unfolding of the whey protein structure exposes reactive sulfhydryl groups, which initiates sulfhydryl–disulfide interchange reactions, resulting in slow polymerization of the adsorbed protein in aged interfacial layers [21]. In contrast, because of their flexible structures, caseins unfold rapidly at the interface and form extended layers up to about 10 nm thick [22]. When the casein molecules are stretched to their maximum extent, their overall surface protein coverage is less than about 1 mg/m^2 but the presence of excess casein increases the monolayer coverage to a maximum value of 3 mg/m^2. In this situation, the parts of the molecules that are in contact with the interface adopt a more compact conformation and the hydrophilic moieties protrude further from the interface [22].

In some commercial milk protein products (i.e. calcium caseinate, casein micelles, milk protein concentrates and micellar casein), proteins (mainly caseins) are present in various states of aggregation, generally cross-linked through calcium-induced interactions. These aggregated forms of milk proteins also adsorb on to the emulsion droplet surface, but much higher concentrations of protein are required to make stable emulsions under similar homogenization conditions. Because of limited spreading at the interface, much greater surface protein coverage (5–20 mg/m^2) is obtained, compared with the molecular forms of casein and whey protein [23,24]. The formation of thick, dense adsorbed layers contributes to the long term stability of emulsions against coalescence by both electrostatic mechanisms and steric stabilization mechanisms.

As well as milk proteins, several plant proteins have also been studied as alternative sources of food emulsifiers. Among the available plant protein sources, the emulsifying properties of the fractions extracted from cereals (α-gliadin from wheat) and legumes (pea albumin and globulins from pea and soybean) [25–30] have been studied. These materials are not discussed in this review.

Oil-in-water emulsions are thermodynamically unstable because of various types of physical and chemical processes. These processes lead to changes in the droplet size distribution and/or the emulsion structure and are referred to as coalescence, flocculation, creaming and Ostwald ripening. During flocculation and creaming, the emulsion structure changes, whereas the droplet size distribution may remain unaltered. In contrast, coalescence and Ostwald ripening lead to changes in the droplet size distribution with time [12–14,31]. Chemical instability includes changes in the composition of the emulsion droplets themselves, such as oxidation and hydrolysis [32]. The ability of proteins to stabilize emulsions comes from electrostatic interactions and steric factors, with a much larger contribution from the steric stabilization mechanism. Protein-based emulsions are generally stable to coalescence over prolonged storage, but, at a low protein to oil ratio, the adsorbed protein molecules/particles are shared by two or more droplets, causing bridging flocculation. This type of flocculation is commonly observed in emulsions formed with aggregated protein products, such as calcium caseinate or micellar casein, in which the droplets are bridged by casein aggregates or micelles. At very high protein to oil ratios, the presence of excess, unadsorbed protein may lead to depletion flocculation in certain types of emulsions. Interactions between droplets stabilized by proteins may be influenced by the presence of certain ions, particularly calcium, because proteins are capable of binding ions.

Sodium-caseinate-stabilized emulsions have been shown to undergo depletion flocculation at protein concentrations above 3.0 wt.%. This has been considered to be due to the change in the aggregation state of sodium caseinate with increasing protein concentration; casein molecules self-associate to form small aggregates in the aqueous phase above a certain critical concentration. In contrast, emulsions formed with whey proteins do not show depletion flocculation, probably because the molecular size of whey proteins is less than the optimum to induce depletion flocculation [24].

The aggregation state and the flexibility of protein molecules can be altered by changes in pH, the addition of divalent cations and the preheating of protein solutions prior to emulsification. These alterations inevitably influence the amount of adsorbed protein, the kinetics of adsorption and the structure of the formed layers. The stability of the resulting emulsions is affected by these changes. The exact

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