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Emulsion stabilisation using polysaccharide–protein complexes

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ABSTRACT

There is a great deal of interest in the Food Industry in the use of polysaccharides and proteins to stabilise oil-in-water emulsions and there is a particular interest nowadays in the use of polysaccharide–protein complexes. There are three classes of complexes namely; (a) naturally-occurring complexes in which protein residues are covalently attached to the polysaccharide chains as is the case, for example, with gum Arabic; (b) Maillard conjugates, which are formed by interaction of the reducing end of a polysaccharide with an amine group on a protein forming a covalent bond; and (c) electrostatic complexes formed between a polysaccharide and a protein with opposite net charge. This review sets out our current understanding of the nature of these different polysaccharide–protein complexes and their ability to stabilise oil-in-water emulsions.

1. Introduction

Proteins have amphiphilic characteristics and as such are able to adsorb strongly at the oil–water interface. They are commonly used in the Food Industry as emulsifiers in the stabilisation of oil-in-water emulsions [1–5]. The amount adsorbed and the conformation adopted at the oil–water interface will depend very much on the protein amino acid composition since adsorption occurs through hydrophobic groups present within their structure. Once adsorbed, the molecules can unfold in order to maximise the number of hydrophobic groups that are in contact with the surface enabling the hydrophilic groups to re-arrange and protrude away from the surface into the aqueous phase. Interaction can sometimes occur between adjacent adsorbed protein molecules through hydrophobic bonding or disulphide bond formation leading to the formation of a viscoelastic layer at the oil–water interface as has been demonstrated by surface rheology and atomic force microscopy [6•].

The adsorbed protein molecules are able to stabilise emulsions by preventing droplet aggregation and coalescence through electrostatic and/or steric repulsive forces [1,2,4,5]. However, they have limitations in so much that, at the protein isoelectric point, the protein molecules will have a net zero charge and unless the adsorbed layer thickness is large enough, droplet aggregation will occur. A further cause of instability in oil-in-water emulsions is Ostwald ripening which becomes particularly important for emulsions containing water soluble oils, for example, flavour oils such as limonene which is used in beverages [3,5]. This phenomenon occurs as a consequence of that fact that the solubility of the oil increases as the size of the droplet decreases. The higher concentration of dissolved material surrounding the smaller droplets results in a concentration gradient and so the dissolved molecules move from the smaller droplets to the larger droplets giving rise to an overall increase in droplet size. The most widely used emulsifier for the stabilisation of flavour oil-in-water emulsions is gum Arabic and its effectiveness is most likely due to the fact that it forms a thick adsorbed layer as will be discussed in more detail below. Gum Arabic is a complex polysaccharide that contains certain fractions which are rich in protein and it has been shown that the protein is covalently attached to the polysaccharide moieties [7]. Currently, there is a considerable interest in identifying other naturally-occurring polysaccharide–protein complexes and the material that has received most attention in recent years is pectin and in particular, sugar beet pectin [8–10•,11•–14]. The concept that polysaccharide–protein complexes can stabilise oil-in-water emulsions has led to the development of gum Arabic look-alike molecules, that is, polysaccharide–protein conjugates formed through the Maillard reaction. This area of study was initiated by Kato et al. and is still an active field of research today [15•–17]. A further area that has received considerable attention in recent years is the use of polysaccharide–protein electrostatic complexes as emulsifiers particularly in the encapsulation of active compounds [18•,19•–21]. The complexes are formed between anionic polysaccharides and proteins at pH values below the protein isoelectric point where they carry a net positive charge. One approach, first reported by Bungeberg de Jong [22], is to use polysaccharide–protein coacervates as emulsifiers [23] but more recent interest has been given to the use of soluble electrostatic complexes [24•,25]. A second approach is to form an emulsion using a protein as the emulsifier and then add

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the polysaccharide which adsorbs onto the protein layer to form a secondary layer on top [26].

2. Naturally-occurring polysaccharide–protein complexes

Gum Arabic is commonly used as an emulsifier in the production of flavour oil-in-water emulsions for application in the Beverage Industry [27–30]. It is a tree gum exudate obtained from the stems and branches of acacia trees (*Acacia senegal* and *Acacia seyal*) which grow across the Sahelian belt in Africa, notably in Sudan, Chad and Nigeria. It is a complex, highly branched polysaccharide consisting of a core of β-1,3 linked galactose residues with branches consisting of galactose, arabinose, rhamnose and glucuronic acid. It has also been shown to contain a small amount of protein (~2.5% for gum from *A. senegal*) as an integral part of its structure. It is now recognised that the gum consists of three main fractions which differ mainly in their molecular size and protein contents. The bulk of the gum (90%) is referred to as the arabinogalactan fraction (AG). It has a molecular mass of 2–3 × 10^5 g/mol and contains very little protein and has been reported to have a disc-like structure [31]. The second major component is referred to as the arabinogalactan-protein fraction (AGP) which represents ~10% of the gum. The AGP has a molecular mass of ~1–2 × 10^6 g/mol and contains about 10% protein. It consists of a polypeptide chain of ~250 amino acids with blocks of carbohydrate with molecular mass of ~4 × 10^4 g/mol covalently attached through serine and hydroxyproline residues [26]. The third minor fraction, referred to as the glycoprotein fraction (GP) represents ~1% of the total gum. It has a molecular mass of ~2 × 10^5 g/mol and contains 20–50% protein but little is known about its structure. The importance of the proteinaceous components within the gum to the emulsification properties was first recognised by Randall et al. who demonstrated that protein-rich fractions adsorbed preferentially onto the surface of orange oil droplets and this has since been supported by the work of others [32,33]. Removal of the protein by treatment with proteolytic enzyme greatly reduces the emulsification properties [32,34] which confirms the role of the protein. It is envisaged that the proteinaceous components adsorb onto the surface of the oil droplets and the covalently linked carbohydrate blocks protrude into the aqueous phase and prevent droplet aggregation through both electrostatic and steric repulsive forces (Fig. 1).

Recently Yadav et al. reported that glycosylphosphatidylinositol lipids were present in the AGP fraction and that these also make a contribution to the emulsification properties [35•]. Further work, however, is required to confirm this.

Zeta potential measurements have confirmed that gum Arabic-coated droplets have sufficient negative charge above pH 4 to prevent droplet aggregation through electrostatic repulsions due to the presence of the glucuronic acid groups in the gum Arabic structure [36–39]. However, the zeta potential decreases at lower pH values as the glucuronic acid groups become undissociated and steric repulsive forces predominate. It has been found that the amount of gum Arabic adsorbed onto limonene oil droplets is ~5 mg/m^2 at pH 7.5 and ~6.5 mg/m^2 at pH 3.5 [37]. These values are significantly higher than might be expected for monolayer coverage suggesting that multilayer adsorption occurs. This could arise through electrostatic interaction between adsorbed and non-adsorbed protein and polysaccharide components of the gum Arabic molecules. It has been shown by rheological measurements that gum Arabic molecules have a tendency to self associate in solution [40] which supports this hypothesis. As noted above, the bulk of the gum (i.e. the AG component) has very little protein associated with it and hence does not play any significant role in the emulsification process.

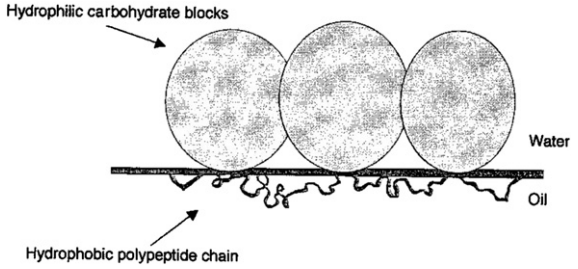

Fig. 1. Schematic representation of gum Arabic adsorbed onto oil droplets.

There has been considerable interest in the Food Industry in recent years in the use of pectins for the stabilisation of oil-in-water emulsions. Pectin is a complex polysaccharide [41,42] and consists of linear chains of 1,4 linked α-D-galacturonic acid residues, interrupted by 1,2 linked L-rhamnose residues. There are branches, consisting of neutral sugars linked to the rhamnose producing 'hairy regions' along the otherwise 'smooth' galacturonic acid main chain. Akhtar et al. [13] showed that depolymerised citrus pectin with a degree of esterification, DE of 70% and molecular mass of 70 kDa produced very stable rapeseed oil emulsions with comparable properties to emulsions prepared with gum Arabic. However, the pectin was not as effective at stabilising limonene oil-in-water emulsions. The supernatant following emulsification was analysed and it was concluded that the material adsorbed was rich in protein. There has been particular interest in recent years in the use of sugar beet pectin as an emulsifier. Sugar beet pectin constitutes ~20% of sugar beet pulp which is obtained as a by-product during the extraction of sugar and in the EU amounts to ~400 K tonnes p.a. A distinct feature of sugar beet pectin is that it contains ferulic acid which is not the case for citrus pectins. Leroux et al. investigated the interfacial and emulsification properties of citrus and sugar beet pectins and found that they both significantly reduced the interfacial tension [9]. The values for 2% pectin solutions at the paraffin oil/water interface were 31.3 mN/m for citrus pectin and 19.4 mN/m for sugar beet pectin. Emulsions were prepared using orange oil and rapeseed oil and the pectin remaining in the aqueous phase was recovered by precipitation and analysed. It was found that the fraction that adsorbed onto the oil contained a higher proportion of protein and had a higher acetyl content than the material in the aqueous phase. De-acetylation did not cause any significant loss in emulsifying capacity and it was concluded that the emulsifying properties were most probably due to the protein components present. Chee Kiong and Williams [10•] determined adsorption isotherms for the adsorption of sugar beet pectin onto limonene oil droplets. It was found that the amount adsorbed was ~9.5 mg/m^2 which is significantly more than might be expected from monolayer coverage as is the case for gum Arabic as discussed above. Furthermore the adsorbed fraction contained 14.7% protein and 2.1% ferulic acid whilst the pectin sample as a whole contained 2.7% protein and 1.06% ferulic acid thus confirming that protein and ferulic acid-rich components adsorbed preferentially. In a further study Chee Kiong et al. reported that the thickness of the sugar beet pectin layer adsorbed onto a model substrate (polystyrene latices) was 140 nm at plateau coverage which was found to be roughly equivalent to the hydrodynamic diameter of the pectin molecules themselves [11•]. The thickness was found to be sensitive to the pH and a value of 107 nm was obtained at pH 4 for a surface coverage of 20 mg/m^2 whilst a value of 70 nm was obtained at pH 8.8. At pH 4 the proteinaceous moieties are likely to carry a net positive charge and hence could form complexes through interaction with the glucuronic acid residues present and form multilayers. At pH 8.8 the proteinaceous moieties are likely to carry a net negative charge and hence are unlikely to form electrostatic complexes.

Nakauma et al. have compared the emulsification properties of sugar beet pectin, soybean soluble polysaccharide and gum Arabic using medium chain triglyceride, MCT as the oil [38]. They demonstrated that the polysaccharide concentration required to produce the minimum droplet size on the emulsification of 15% MCT was in the order sugar beet pectin (~1.5%) < soybean soluble polysaccharide (~4%) < gum Arabic (~10%). Whilst they found that the amount