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Insignificant impact of the presence of lactose impurity on formation and colloid stabilising properties of whey protein–maltodextrin conjugates prepared via Maillard reactions

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ABSTRACT

We investigate the sensitivity of steric stabilising properties of protein–polysaccharide conjugates, prepared via the Maillard reactions, to the presence of sugar impurity during synthesis. The sugar can also react with protein, thus rendering potential sites on protein unavailable for linkage with polysaccharide and severely reducing the efficiency of producing these types of food dispersants. We demonstrate that despite the presence of a relatively high molar ratio of lactose contaminant to maltodextrin (10:1), the covalent complexes between maltodextrin DE19 (MD19) and Whey Protein Isolate (WPI) are still formed and continue to show superior emulsifying and colloid stabilising properties compared to native protein. The improvement was particularly marked under unfavourable environmental conditions, such as pH ~ pI of protein, up to a storage time of 21 days. In contrast, the covalent complexes of lactose + WPI were found to have rather poor emulsion stabilising characteristics, under the same conditions. We also confirm this result by performing theoretical Self Consistent Field type calculations. The stability of emulsions was monitored using a variety of measures including the average droplet size (ADS), droplet-size distribution (DSD), rheological flow behaviours and confocal laser imaging microscopy. The suggestion that the WPI-MD19 (1:2, w/w) system is quite tolerant to the presence of lactose is of significance in future large scale industrial manufacturing of such food dispersants, due to less stringent requirements for the purity of raw material (WPI).

1. Introduction

Food proteins have been emulsifiers and colloid stabilisers of choice in many food colloid formulations. Proteins are surface active by the virtue of their amphiphilic nature. When proteins are adsorbed at the oil-water interface they contribute to both of the two major means of protecting emulsion droplets against flocculation and coalescence. The two mechanisms in mind are the electrostatic and steric stabilisations of colloids (Dickinson, 2010). However, these stabilising properties can be detrimentally affected, or even completely eliminated, under the influence of certain environmental conditions, such as pH, being too close to the pI of proteins, or at high ionic strength (McClements, 2015; Walstra, 2003). In order to maintain and improve the emulsifying and stabilising properties of proteins under these unfavourable conditions, native proteins are covalently bonded to polysaccharide molecules (Akhtar & Dickinson, 2007; Dickinson & Semenova, 1992). The fundamental concept underlying this modification is to synthesise a conjugate which can behave as a stabilising agent with considerably enhanced steric stabilising property, thus not overly sensitive to changes in pH or to background electrolyte concentration. The hydrophobic segments of protein can strongly adsorb at the oil-water interface, while the hydrophilic regions (i.e. now mainly polysaccharides) protrude away from the interface, thus providing a thick surface layer and improved steric stabilisation (Dickinson, 2015).

One of the simple methods to prepare the conjugates is by heat treatment to induce the necessary Maillard reactions between the protein and polysaccharide. This has to be achieved under careful controlled conditions, such as relative humidity, temperature and the processing time. This can be done either in powdered state or in an aqueous solution (Aoki et al., 1999; Dickinson & Galazka, 1991; Kato & Kobayashi, 1991; Kim & Shin, 2015; Qi, Liao, Yin, Zhu, & Yang, 2010; Zhu, Damodaran, & Lucey, 2010). Moreover, the Maillard-type conjugates can be prepared under high pressure (Xu et al., 2010), by microwave heating (Guan, Qiu, Liu, Hua, & Ma,

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2006), or using pulsed electric fields (Sun, Yu, Zeng, Yang, & Jia, 2011). Almost all of the previous studies in literature have suggested that the emulsifying and stabilising properties of various food proteins are improved significantly through such Maillard reactions with a polysaccharide. Furthermore, these conjugates also exhibit other potential applications as foaming agents and heat-set gelling agents (Martinez-Alvarenga et al., 2014; Spotti et al., 2013a, 2013b). The overall conclusion of these studies has been that the conjugates, prepared in this manner, are excellent emulsifiers and stabilisers with significant possible potential for use in food industry. This is especially the case as their synthesis involves no additional chemicals and due to their relatively simple, if not exactly cheap, preparation method (Kato, 2002).

Before conjugates can be produced on an industrial scale, it is necessary to investigate a number of crucial factors which can significantly influence the required functionalities of these complexes. Examples are the ratio of protein to polysaccharide, processing conditions, and the molecular weights of polysaccharides used, but to name a few (Oliver, 2011). It has been reported that the emulsifying and stabilising properties of conjugates have a positive correlation with the length of polysaccharides grafted on the protein (Shu et al., 1996). This finding supports the stabilising model of conjugates which was first proposed by Dickinson and Semenova (1992). Based on the evidence, it can be predicted that proteins attached to high-molecular-weight (HMW) polysaccharides such as maltodextrin should have better stabilising properties than those modified by low-molecular-weight (LMW) sugars, like lactose under the same processing conditions. Similar conclusions are also supported by theoretical considerations where it has been shown that the attachment of short polysaccharide chains, depending on the location of grafting, can result in conjugates with an inferior stabilising property when compared to protein on its own (Akinshina, Ettelaie, Dickinson, & Smyth, 2008). It may be expected then, that if both HMW and LMW polysaccharides exist during the Maillard reaction, the stabilising properties of the conjugates may lie somewhere between that of the complexes resulting from grafting by HMW polysaccharides and ones produced by the Maillard reaction involving LMW sugars. The aim of the current work is to find out at what level of lactose impurity, unavoidable in commercial whey protein, it is possible to produce conjugates with interfacial properties comparable to those prepared by the Maillard reaction between pure WPI and maltodextrin. To evaluate the critical molar ratio of lactose, we have chosen the whey protein isolate-maltodextrin DE19 (WPI-MD19) conjugate as a model system. This conjugate is known to have excellent emulsifying and stabilising properties, as has been demonstrated in our previous studies (Akhtar & Dickinson, 2003, 2007).

The paper is organised as follows. In the next section we describe our method for preparation of conjugates, that of emulsions and the evaluation of the emulsion stability. The results are presented next and discussed in the light of our current understanding of the interfacial behaviour of covalently bonded protein + polysaccharides. We provide a few preliminary theoretical calculations based on Self Consistent Field theory (SCF), along the lines used in our previous work, to compare the emulsion steric stabilising properties of WPI + MD19 with those of WPI + Lactose (Akinshina et al., 2008; Ettelaie & Akinshina, 2014; Ettelaie, Akinshina, & Maurer, 2012). These calculations are useful in lending further support to conclusions drawn from the experimental observations.

2. Materials and methods

2.1. Materials

The lactose-free whey protein isolate powder was offered by Davisco Foods International (USA). The lactose was purchased from Fisher Scientific Ltd., and maltodextrin DE19 (M_w = 8.7 kDa) was offered by Roquette (UK) Ltd. The sunflower oil was purchased from local supermarket Morrison (Leeds, UK). Other chemicals and reagents used in this project are of analytical grade.

Table 1
Recipe of WPI-MD19 conjugate with various molar ratios of lactose before the Maillard reaction.

WPI (g)	MD19 (g)	Lactose (g)	MD19: lactose (molar)[a]
1	2	0.0787	1:1
1	2	0.1574	1:2
1	2	0.3135	1:4
1	2	0.4703	1:6
1	2	0.7839	1:10

[a] MD19 (M_w = 8.7 kDa); Lactose (M_w = 342.3 g/mol).

2.2. Conjugates preparation

The whey protein isolate (WPI) and maltodextrin DE19 (MD19) were fully dissolved in 100 ml distilled water with gentle stirring under room temperature. Various recipes involving different ratios of MD19 to lactose content, were prepared as shown in Table 1. The solutions were stored in the fridge (at 4 °C) overnight and then frozen at −30 °C for 3 hours. These were freeze dried for a period of 24 h. After collection, the resulting powder of WPI and MD19 (and lactose were appropriate) was placed in a pre-heated desiccator under 80 °C for 3 h, with relative humidity controlled by saturated KBr solution. The complex of WPI and MD19 was stored in a dark and dry place for further application. The conjugates of WPI-MD19 (1:2, w/w) and WPI-Lactose (2:1, w/w) were similarly prepared as controls.

2.3. Degree of conjugation

The degree of conjugation (DC) of each protein–polysaccharide complex after the Maillard reaction was determined by o-phthalaldehyde (OPA) tests. The OPA reagent was prepared based on the previous literature (Nielsen et al., 2001). Each conjugate was dissolved into distilled water with gentle stirring at a concentration corresponding to a WPI content of 1.0 mg/ml. For each prepared solution, 0.4 ml of the sample was added to 3 ml OPA reagent, mixing on a Topmix at 1600 rpm for 5 seconds. The mixture was allowed to stand for exactly 2 min at room temperature before its absorbance at a wavelength of 340 nm was measured using a spectrophotometer. The baseline was established by untreated pure WPI solution. The degree of conjugation for this complex can thus be calculated as follows:

$$\text{Degree of conjugation (DC)}\% = \frac{(C_{\text{WPI}} - C_{\text{nConj}}) \times 100\%}{C_{\text{WPI}}}$$

where C_{WPI} is the concentration of native WPI and C_{nConj} is the concentration of unreacted WPI in the conjugate sample. The analysis of each sample was carried out in triplicate.

2.4. O/W emulsion preparation

Before homogenisation process, the aqueous buffer (500 ml) at ionic strength 0.1 M and pH of 2.9, was prepared by mixing citric acid (3.125 g) and sodium chloride (2.920 g) into distilled water. Sodium azide was also added to the aqueous buffer at a concentration 0.1% (w/v), as a preservative. The appropriate amount of protein–polysaccharide conjugate was dissolved into the aqueous buffer by gentle stirring at room temperature. This concentration was chosen so as to ensure a protein (WPI part of conjugates) concentration of 2% (w/v), taken account of the fact that conjugated biopolymers have a considerably larger molecular