BEHAVIOUR OF EMULSIONS STABILIZED BY MAILLARD-BASED GLYCOCONJUGATES UNDER SIMULATED GASTROINTESTINAL CONDITIONS

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ABSTRACT

There is much interest in controlling lipid digestibility within the human gastrointestinal tract. Particularly, there is great interest in modulating the functionality of emulsions which are suitable for the delivery of lipophilic ingredients. Various recent studies have shown it is possible to harness the natural Maillard reaction to fabricate novel emulsifiers which can enhance and modulate emulsion stability and functionality. This work focused on the behaviour of oil droplets coated by protein-carbohydrate Maillard conjugates under simulated upper gastrointestinal tract (GIT) conditions.

A variety of functional glycoconjugates were produced through dry heating (60°C, 24 h, 79% RH) of b-lactoglobulin with dextrans with different molecular weights. These were used as emulsifiers to produce corn oil-in-water emulsions by high pressure homogenization. Laser based particle-sizing and ζ-potential measurements combined with in vitro simulated digestion models were used to study the effects of pH, bile concentration, pepsin and pancreatic lipase on emulsion stability. Our results indicate that increasing the molecular weight of the dextran moieties attached to the β-lactoglobulin molecules increased emulsion physical stability to gastric conditions (up to 2 h) and slightly decreased emulsion susceptibility to small intestinal digestion processes (bile adsorption and lipase digestion), which was attributed to an increase in steric repulsion. Overall, the results suggest that such natural conjugates could be useful for improving emulsion stability, controlling fat digestion, and delivering lipophilic ingredients to the small intestine and colon.

Keywords: Emulsions, Maillard conjugates, protein digestion, lipid digestion

INTRODUCTION

In face of the rising obesity levels in western countries, much attention has been dedicated to controlling the digestibility of lipids within the human gastrointestinal tract [1-3]. Particularly, there is great interest in rationally designing the functionality of lipids and emulsions [1, 3-5]. Previous studies have shown that Maillard conjugates are effective at controlling emulsion and lipid stability and digestibility [6-8]. These studies established the influence of various reaction conditions on the formation and properties of these conjugates, e.g. temperature, time or protein-to-carbohydrate ratio [8]. Others have even shown that Maillard conjugates may reduce protein allergenicity [9].

This study focused on the impact of β-lactoglobulin-dextran Maillard conjugates on emulsion behaviour under gastric and small intestine conditions. Such conjugates have been shown to be better emulsifiers than β-lactoglobulin alone endowing emulsion droplets with improved stability [8, 10-14]. Increasing the molecular weight of the dextran moieties within the conjugates has been found to improve emulsion stability by increasing the thickness of the steric layer around the droplets [11].

Recently it has been shown that emulsification alters the gastrointestinal proteolysis of b-lactoglubulin and that glycosylation decreases its susceptibility to gastrointestinal digestion [15, 16]. Based on these and other publications, we sought to establish some of the principles for the design of b-lactoglobulin Maillard conjugates to modulate emulsion digestion in the human gastrointestinal tract (GIT). Thus, we produced different conjugates varying only in the MW of the dextran moieties and used them to stabilize corn oil in water emulsions. Adopting a bottom up approach, emulsion formulations were subsequently examined for the influence of pH, pepsin activity, bile and pancreatic lipase under simulated GIT conditions.

MATERIALS & METHODS

Materials. Food grade β-lactoglobulin (Davisco Foods International Inc., Le Sueur, MN) and Dextrans from Leuconostoc spp. with molecular weights of ~10kDa, 40kDa and 188kDa (Sigma Chemical Co., St. Louis, MO) were used. Corn oil was purchased from a local supplier and all ingredients were stored in a refrigerator prior to use. Pepsin, porcine bile extract, pancreatic lipase (Type II porcine) and all other reagents were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO) or Fisher Scientific (Pittsburg, PA).

Preparation of Maillard-based conjugates and corresponding Emulsions. Maillard conjugates were prepared based on an adjusted protocol of that previously described [11]. Basically, physical mixtures of b-lactoglobulin (b-lg) and dextran (D) were lyophilized and subsequently 'dry heated' (24 h, 60 °C, 76% RH) before being used as emulsifiers. Mixtures were labeled as b-lg-D(number) mixtures or conjugates, where the numerical index points to the dextran's MW. Oil in water emulsions containing 5% oil (w/w) and 1% (w/w) protein-based emulsifier were produced through high-pressure homogenization (Microfluidizer M-110L processor, Microfluidics Inc., Newton, MA) at 82 MPa as recently described [9, 17-19]. The homogenizer was chilled throughout the homogenization process and emulsions were subsequently stored in a refrigerator until analysis within 3 days of production.

Laser-based droplet characterization. The mean volume-weighed diameters ($d_{4,3}$) of the emulsion droplets and the particle size distribution were obtained using a Malvern Mastersizer 2000 (Malvern Instruments, Worcestershire, U.K). The electrokinetic charge (ζ-potential) of the oil droplets was obtained using a capillary electrophoresis device inserted into a dynamic light scattering instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, U.K.).

Exposure to gastric conditions. As gastric pH and pepsin induce significant changes in emulsion characteristics [20, 21], their influence on droplet size was studied. Specifically, emulsions were incubated at 37°C for 2 h in simulated gastric fluid (SGF) containing pepsin, as recently described [21].

Exposure to small intestine conditions. As the process of emulsion digestion involves various events with bile salts' adsorption to droplet interfaces and pancreatic lipase digestion being two important phenomena. Therefore we examined the effect of physiological levels of bile on emulsion characteristics after incubation for 2 h at 37°C. The lipase digestibility of the emulsions was determined using an in vitro small intestine model in which the free fatty acid release is closely monitored using a pH-STAT instrument (Titrando, Metrohm, Switzerland), as recently reported [17, 19, 22, 23].

RESULTS & DISCUSSION

Accumulating evidence show that emulsifier and interfacial modifications of lipid droplets offer the possibility to modulate emulsion overall stability and possibly emulsion performance in the GIT [1, 3, 4, 7, 8]. In this study we sought to establish a link between the characteristics of b-lg-dextran Maillard conjugates, i.e. dextran molecular weight, to the potential functionality of emulsions in the upper human gastrointestinal tract. In the first stage of the work we characterized the droplet size and ζ-potential of emulsions made with pure b-lg, b-lg-dextran physical mixtures and b-lg-dextran conjugates (**Figure 1**).

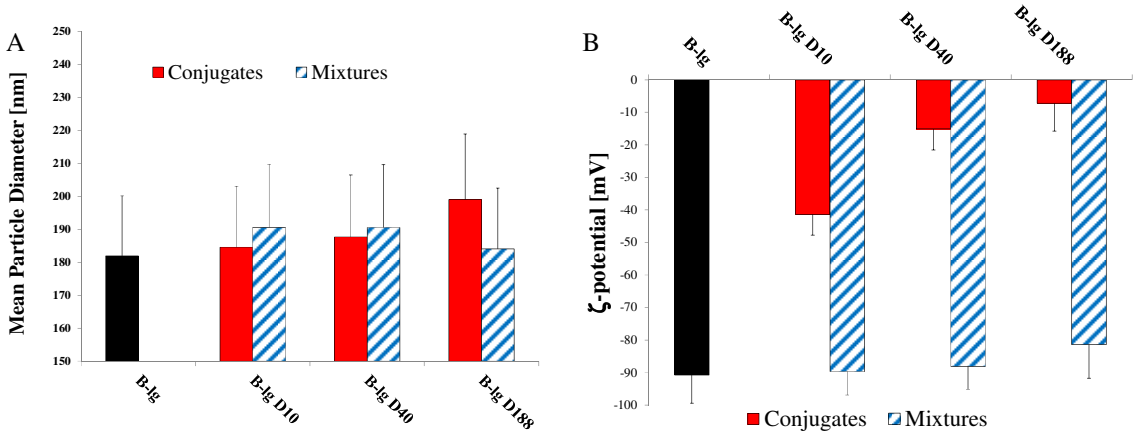

Figure 1. Characteristics of oil in water emulsions stabilized by either physical mixtures of β-lactoglobulin (B-lg) and dextrans with molecular weights of 10kDa (D10), 40kDa (D40) and 188kDa (D188) or their corresponding Maillard-based conjugates. [A] Mean particle diameter $d_{4,3}$ and [B] ζ-potential.

As can be seen in **Figure 1[A]**, mean droplet sizes were not significantly affected by the presence of dextrans, either in physical mixtures or conjugates. However, ζ-potential measurements (**Figure 1[B]**) revealed that the addition of dextrans decreased the ζ-potential of emulsions, with emulsions stabilized by conjugates having a significantly higher ζ-potential values, i.e. less charged droplets. Moreover, it was found the molecular weight of the dextrans appeared to be related to the changes in emulsion ζ-potential. Overall, it was found that increasing the molecular weight of the dextran moiety in the conjugate increased the emulsion ζ-potential suggesting droplet interfaces to be less charged. Wooster et al (2006)[11] found that increasing dextran MW increased the thickness of the interfacial layer surrounding droplets. Therefore, we believe that the soluble dextran moieties covalently bound to the protein thicken the surrounding layers around the droplets which provide a screening effect. Consequently, the charged droplet surfaces are distanced and secluded from interactions with the continuous phase. As a result, the electrophoretic mobility of the droplets is altered and an apparent decrease in ζ-potential is observed. These altered emulsion characteristics could affect emulsion responsiveness to environmental stressors and interfacial reactions which were the focus of the following experiments.

Emulsion response to gastric conditions

The stomach is a major bioreactor in which food is digested through exposure to highly acid conditions, proteolytic activity and agitation [3, 20, 21, 24]. Moreover, it has been shown that gastric conditions have a considerable destabilizing effect on emulsions [20, 21]. We therefore challenged emulsions versus pH and in simulated gastric conditions with pepsin. First, emulsion stability to pH was tested. These experiments were based on previous reports showing protein-stabilized emulsions are known to be unstable at pH values close to the protein's isoelectric point. We therefore determined the mean droplet diameters of emulsions at the pH of production (pH 7.0) and at pH 5.0 (**Figure 2**), close to b-lg isoelectric point (pH 4.8-5.2).

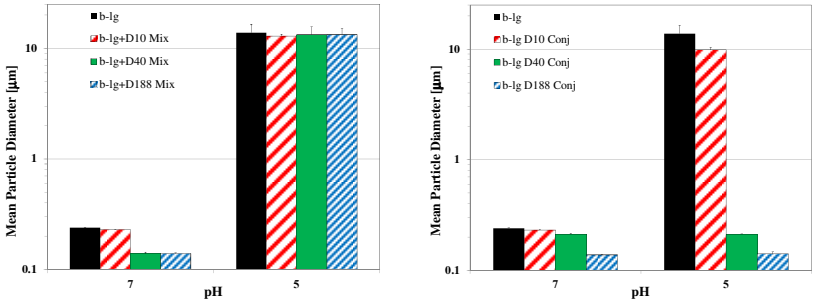

Figure 2. Influence of pH on the mean particle diameter [$d_{4,3}$] of oil in water emulsions stabilized by [A] physical mixtures of β-lactoglobulin (b-lg) and dextrans with molecular weights of 10kDa (D10), 40kDa (D40) and 188kDa (D188) or their corresponding Maillard conjugates [B].

As can be seen in **Figure 2[A]** emulsions stabilized by physical mixtures lost their stability at pH 5.0 where a significant change in droplet size was observed. In contrast, **Figure 2[B]** shows that emulsions stabilized by Maillard conjugates with dextrans of 40kDa and 188kDa successfully maintained their original sizes (pH 7.0). These results pointed to an altered responsiveness of conjugate-stabilized emulsions to environmental stressors. We therefore challenged conjugate-stabilized emulsions to gastric conditions by mixing them with simulated gastric fluid and pepsin. Following 2 h of incubation in simulated gastric condition the laser scattering of the samples was measured and the particle size distribution (PSD) curves plotted (**Figure 3**).

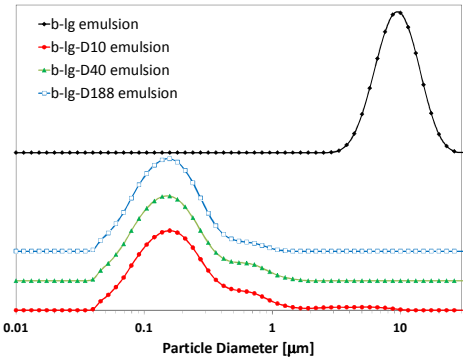

Figure 3. Volume-weighted particle distribution curves of oil in water emulsions stabilized by conjugates of β-lactoglobulin (b-lg) and dextrans with molecular weights of 10kDa (D10), 40kDa (D40) and 188kDa (D188) after 120min of pepsin digestion under simulated gastric conditions.

Following proteolysis under gastric conditions b-lg-stabilized emulsions exhibited a significant increase in size from sub-micron sized droplets (**Figure 1[A]**) to droplets with a mean $d_{4,3}$ diameter of 9.335±0.123µm. These results coincide with a recent work which linked the phenomenon to proteolysis of the droplet coating and droplet coalescence [21]. **Figure 3** also shows that no significant changes in size were noted in the conjugate-stabilized emulsions and the mean sizes were kept in the sub-micron range. This is likely to arise from decreased proteolysis under gastrointestinal conditions possibly due to emulsification and glycation which have been recently reported to alter the susceptibility of food proteins such as b-lg [15, 16, 25]. Practically, the results imply that the properties of emulsion digesta leaving the stomach into the small intestine would have different characteristics, e.g. droplet size, composition and interfacial area. In turn these are expected to affect relevant physiological processes, like bile adsorption and lipid digestion by pancreatic lipase.

Emulsion response to small intestine conditions

In light of the simulated gastric digestion findings, it was important to understand the 'down-stream' response of conjugate-coated emulsions to bile at physiological levels and lipase digestion. The effects of bile addition on the electrokinetic charge (ζ-potential) of all conjugate-coated emulsions are given in **Figure 4.**

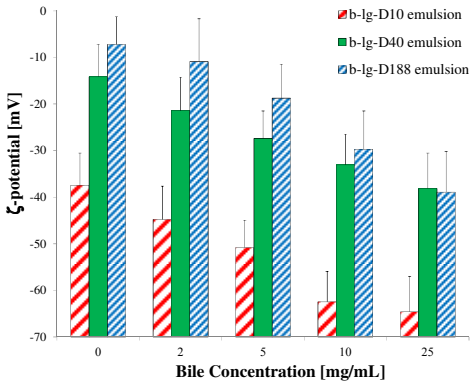

Figure 4. Electrokinetic charge (ζ-potential) of emulsions stabilized by conjugates of β-lactoglobulin and dextrans with molecular weights of 10kDa (D10), 40kDa (D40) and 188kDa (D188) incubated for 2 h at 37°C with increasing levels of bile.

Previous studies show bile addition alters ζ-potential of emulsions due to protein displacement from the droplet interfaces [17, 19, 26]. However, the results given in **Figure 4** show that even excess of bile (25mg/mL) not all emulsions reached the same final ζ-potential values and that these values do not fit values found previously for bile-stabilized emulsions [27]. Hence, emulsions stabilized by b-lg-D10 are expected to have full displacement of the emulsifier while it is not likely to be the case for b-lg-D40 and b-lg-D188 stabilized emulsions.

In the next experiments we examined the lipase digestibility of the conjugate-coated emulsions using an established *in vitro* digestion model designed to simulate the small intestine [23, 28, 29]. The profiles of free fatty acids (FFA) released *versus* digestion time of a b-lg-coated emulsion and samples are given in **Figure 5**.

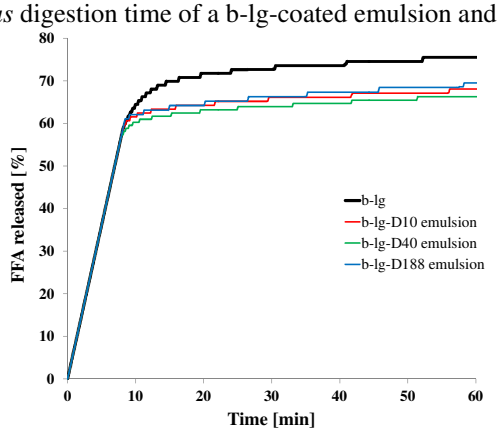

Figure 5. Release of free fatty acids by pancreatic lipolysis of oil in water emulsions stabilized by conjugates of β-lactoglobulin and dextrans with molecular weights of 10kDa (D10), 40kDa (D40) and188kDa (D188).

The results in **Figure 5** show that although the initial rate of digestion was similar for all emulsions the final extent of digestion was reduced for conjugate-stabilized emulsions compared to the b-lg stabilized control. Contrary to the previous experiments, the molecular weight of the dextran moiety did not seem to have a significant effect on the rate or extent of FFA release. Based on our findings described herein one could hypothesize that inefficient displacement of the conjugates from the droplet interfaces (implied by the data in **Figure 4**) could hinder lipase adsorption and/or lipolytic activity under small intestine conditions. However, more advanced GIT models and further experiments, are required to test this hypothesis and to better approximate the performance of conjugate-stabilized emulsions in the human gastrointestinal tract.

CONCLUSION

Overall, this study examined the impact of natural Maillard-based b-lactoglobulin dextran conjugates on the behaviour of emulsions under simulating GIT conditions. Data presented shows that the conjugates did in fact affect emulsion characteristics and responsiveness to pH, gastric pepsin and bile. Conjugates made with dextran with low MW (10kDa) were found to have the least effect on emulsions compared to the native protein. The overall observed effects are believed to arise from increased steric and/or Van der Waals repulsion between droplets originating from the dextran moieties bound to the protein emulsifier. Interestingly, we found that conjugate-coated emulsions exhibit enhanced physical stability to gastric conditions and altered lipase digestibility using an *in vitro* digestion model. These results suggest that emulsions with good physicochemical stability and modulated lipid digestibility could prospectively be fabricated using natural Maillard-based conjugates. Further research is needed to establish the principles for the rational and safe design of Maillard-based conjugates for optimizing emulsion performance in the human gastrointestinal tract and potentially controlling fat digestion and targeting the delivery of bioactive lipophilic ingredients.

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