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Influence of oat components on lipid digestion using an *in vitro* model: Impact of viscosity and depletion flocculation mechanism

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ABSTRACT

Depletion flocculation is a well-known instability mechanism that can occur in oil-in-water emulsions when the concentration of non-adsorbed polysaccharide exceeds a certain level. This critical flocculation concentration depends on the molecular characteristics of the polysaccharide molecules, such as their molecular weight and hydrodynamic radius. In this study, a range of analytical methods (dynamic shear rheology, optical microscopy, and static light-scattering) were used to investigate the interaction between lipid droplets and polysaccharides (guar gum and β-glucans) of varying weight-average molecular weight and hydrodynamic radius, and concentration. The aim of this work was to see if the health benefits of soluble fibers like β-glucans could be explained by their influence on the structure and digestibility of lipid emulsions. The apparent viscosity of the emulsions increased with increasing polysaccharide concentration, molecular weight, and hydrodynamic radius. Droplet flocculation was observed in the emulsions only at certain polysaccharide concentrations, which was attributed to a depletion effect. In addition, the water-soluble components in oat flakes, flour, and bran were extracted using aqueous solutions, to examine their impact on emulsion stability and properties. Then, the rate and extent of lipolysis of a sunflower oil-in-water emulsion in the presence of these oat extracts were monitored using the pH-stat method. However, the inhibition of lipolysis was not linearly related to the viscosity of the oat solutions. The water-soluble extracts of β-glucan collected from oat flakes had a significant inhibitory effect on lipolysis. The results of this study increase our understanding of the possible mechanisms influencing the impact of oat constituents on lipid digestion. This work also highlights the importance of considering the molecular properties of polysaccharides, and not just their impact on solution viscosity.

1. Introduction

The ability of oat (*Avena sativa* L.) to affect lipid metabolism and blood cholesterol levels is now well-known even though the mechanisms involved are not fully understood (Grundy, Fardet, Tosh, Rich, & Wilde, 2018). Oats contain a range of constituents that may positively impact human health, especially water-soluble polysaccharides such as β-glucan (Martínez-Villaluenga & Peñas, 2017; Miller & Fulcher, 2011). This type of polysaccharide may inhibit lipid digestion due to its ability to increase viscosity or promote droplet flocculation, which reduces the access of lipase to the oil droplet surfaces (Bai et al., 2017; Grundy, Quint, Rieder, Ballance, Dreiss, Cross, et al., 2017). Consequently, the presence of these soluble dietary fibres in foods could benefit human health by modulating the blood lipid levels after ingestion of foods rich in lipids. However, there is currently a poor understanding of the precise molecular and physicochemical mechanisms by which dietary fibres inhibit lipid digestion.

In the late 1990s, it was shown experimentally that neutral non-adsorbing polymers could promote droplet flocculation in oil-in-water emulsions through a depletion mechanism (Jenkins & Snowden, 1996). The tendency for depletion flocculation to occur depends on the molecular weight (Mw) and hydrodynamic radius (R_h) of the polymer molecules, which has been described mathematically using theoretical models (Asakura & Oosawa, 1954, 1958). Non-adsorbed polymers induce flocculation in emulsions through an osmotic effect. In an emulsion containing non-adsorbing polymers, there is a region surrounding

Abbreviations: BG1, oat β-glucan of high Mw; BG2, oat β-glucan of medium Mw; BG3, oat β-glucan of low Mw; CFC, critical flocculation concentration; CVC, critical viscosity concentration; FFA, free fatty acids; Mw, weight-average molecular weight; R_h, weight-average hydrodynamic radius; WPI, whey protein isolate

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each droplet where the polymer concentration is depleted (depletion zone). As a result, there is an osmotic pressure between the depletion zone and the bulk polymer solution. It is energetically favourable to minimise the osmotic potential differences in the system, so the system will tend towards a state where the total volume of the depletion zones is minimised. Therefore, when two lipid droplets approach each other so that their depletion zones overlap, there is a reduction in the total volume of solution from which the polymers are excluded, which is energetically favourable. Thus, the system tends towards droplet association and drives flocculation. The magnitude of the osmotic pressure increases with increasing polymer concentration, and so depletion flocculation can happen when the attractive forces outweigh the repulsive forces in the system (McClements, 2000).

Droplet flocculation often promotes more rapid gravitational separation (creaming) in an emulsion because the particle size is effectively increased. However, creaming may not be observed in some cases, because the viscosity of the solution also increases with increasing polymer concentration. For a particular polymer preparation, there is a critical concentration (c^*) above which polymer entanglement occurs and a viscoelastic network is formed that restricts oil droplet movement (Sharafbafi, Alexander, Tosh, & Corredig, 2015; Syrbe, Bauer, & Klostermeyer, 1998). A number of experimental studies showed that the presence of different types of food-grade biopolymers in oil-in-water emulsions can induce depletion flocculation (Chung, Degner, & McClements, 2013; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2014; Minekus et al., 2005). The tendency for droplet flocculation and creaming to take place depends on the molecular characteristics and concentration of the polymers used, and has to be established for different kinds of polymers.

The present study was designed to establish the impact of polymer size and concentration on the viscosity and flocculation of oil-in-water emulsions, using common food-grade neutral polysaccharides (i.e., guar gum and β-glucan) with different molecular characteristics. Guar gum is a well characterised source of galactomannan that we used as a control. On the other hand, the β-glucan was selected because it is one of the main water-soluble polysaccharides found in oat, and has been proposed to be the cause of many of its health benefits, such as prevention of cardiovascular diseases, diabetes, obesity, cancer, and hypertension (Khan et al., 2018; Martínez-Villaluenga & Peñas, 2017; Rebello, O'Neil, & Greenway, 2016; Surampudi, Enkhmaa, Anuurad, & Berglund, 2016). In addition, water-soluble extracts isolated from oat flakes, flour, and bran (BG32) were collected to determine their impact on the stability of emulsions. Finally, we used these soluble extracts as a source of β-glucan and monitored their potential impact on lipid digestion using our *in vitro* duodenal model, in order to obtain some insights into the potential roles of polymer viscosity and depletion flocculation on free fatty acid (FFA) release. This study should provide some valuable insights into the molecular and physicochemical origin of the health benefits of soluble fibres in the human diet and complement some previous studies (Grundy, Quint, Rieder, Ballance, Dreiss, Butterworth, et al., 2017; Grundy, Quint, Rieder, Ballance, Dreiss, Cross, et al., 2017). Our main objective was therefore to fully characterise the materials used in this previous work, while investigating further how they influenced the emulsion stability. We believe that the innovation of the work presented here relies on more detailed characterisation steps that are often missing in the literature.

2. Materials and methods

2.1. Materials and samples characterisation

Sunflower oil, sodium chloride (99.8%), calcium chloride (99%), bovine bile extract, and pancreatin (40 U/mg of solid based on lipase activity) were purchased from Sigma-Adrich (Poole, Dorset, UK). High Mw oat β-glucan (BG1) was a generous gift from Dr Susan Tosh at Agricultural and Agri-Food Canada. Swedish Oat Fiber (Swedish Oat Fiber AB, Bua, Sweden) provided medium Mw β-glucan (BG2, brand name BG90) and the oat bran (brand name BG32). Low Mw oat β-glucan (BG3) was obtained from Megazyme (Bray, Wicklow, Ireland; Product Code: *P*-BGOM). Guar gum flour (Meyprogat M150) was provided by Dr Graham Sworn (Danisco, Paris, France). Oat flakes and oat flour were obtained as previously described (Grundy, Quint, Rieder, Ballance, Dreiss, Cross, et al., 2017). Powdered whey protein isolate (WPI) was donated by Davisco Foods International (Le Sueur, MN, USA).

The methods used for the determination of the moisture content, lipid content, polysaccharide concentrations of the oats (flakes, flour and bran), BG1, BG2, BG3, and guar gum are detailed elsewhere (Grundy, Quint, Rieder, Ballance, Dreiss, Butterworth, et al., 2017). Weight-, number-average molar mass, polydispersity, and weight-average R_h of purified β-glucan and galactomannan were determined by size-exclusion chromatography with a series coupled Wyatt 8 angle multi-angle light scattering detector, followed by a Wyatt Viscostar II viscosity detector, and finally a Wyatt T-rex refractive index detector (SEC-MALS-VISC-RI). For the oats flakes, flour and bran, the β-glucan was directly extracted and purified (omitting protease and xylanase treatment) as described by Rieder, Ballance, and Knutsen (2015). Briefly, 2 mg of purified sample was weighted into a 2 mL Eppendorf tube with screw lid. Twenty μL of 80% aqueous ethanol was added, vortexed, and left for 1 h with occasional mixing. To this 1.5 mL of 0.1 M sodium nitrate containing 0.02% sodium azide was added and the sample placed into a boiling water bath for 5 min followed by shaking at a frequency of $25 s^{-1}$ in a Retch 400 M oscillating mill. This procedure of boiling and shaking was repeated a further time. Samples were finally filtered through a 0.8 μm syringe filter. One hundred μL of each sample was injected via a 100 μL loop onto two size-exclusion chromatography columns coupled in series (Tosho Bioscience TSK-gel PXWL 5000 and 6000). An isocratic mobile phase of 0.1 M sodium nitrate containing 0.02% sodium azide at a flow rate of 0.5 mL/min was used to elute the samples and delivered by a Shimadzu HPLC pump. Data was processed in custom Wyatt Astra software. The second virial coefficient was set at zero and a refractive index increment of 0.146 was used. As positive control, a certified pullulan standard of known molar mass from Polymer Standards Service was used. Treatment of samples/extracts containing β-glucan with lichenase followed by SEC-MALS-VISC-RI eliminated the concentration signal from the refractive index detector used to measure Mw in conjunction with the MALLS detector. This confirmed all the analysed sample/extracts comprised β-glucan. The amount of β-glucan released during the incubation of the oat materials were measured using an enzymatic method based on a cereal mixed-linkage β-glucan kit from Megazyme (Megazyme, Product Code: K-MBGL).

2.2. Preparation of the experimental material

Solutions of guar gum or β-glucan were obtained by slowly sprinkling the polymer powder into a rapidly swirling vortex of 10 mM phosphate buffer, pH 7. The mixture was heated at 80 °C for 2 h before being left at room temperature overnight. This procedure ensured that the polymers were fully hydrated. Additionally to those pure polymer preparations, water-soluble extracts from selected oat materials (flakes, flour or bran with a total β-glucan content of 1.0%, w/v) were incubated in 10 mM phosphate buffer as previously described (Grundy, Quint, Rieder, Ballance, Dreiss, Butterworth, et al., 2017). After 1 or 72 h of incubation, the samples were centrifuged at 1800 g for 10 min and the aqueous phase collected. This aqueous phase is referred as oat extract in the rest of the manuscript. Oat extracts were used in the present work in order to identify if the compounds/structures released during incubation, and not the oat particles, where responsible for the reduced lipid digestibility observed in our previous study (Grundy, Quint, Rieder, Ballance, Dreiss, Cross, et al., 2017). The incubation times were selected because 1 h corresponds to the duration of the

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