FoodPro ForHealth
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Investigating the role of food structure and processing in lipid digestion for production of healthier food

FoodPro ForHealth aimed to investigate the role of the food matrix on the physico-chemical breakdown of emulsions during *in vitro* digestion, in order to develop novel approaches for enhancing satiety and/or reducing dietary fat intake. A multidisciplinary approach combining fundamental physical and biological sciences was required to better understand the mechanisms underlying lipid digestion and the impact of emulsion structure on lipase activity. This proposal involved studies that provided important information about the interaction of food biopolymers with digestive enzymes, surfactants and lipids in the digestive tract. Through improved understanding of this effect, we have developed food systems with potential to modulate the digestion and sustained release of lipids. FoodPro ForHealth focused on ways of limiting the access of bile salts to the lipid surface, as a potential mechanism of slowing lipid digestion, either by designing interfaces that resist displacement by bile salts, or by sequestering bile salts in the duodenum. Understanding how the bile salts interact with emulsifiers and stabilizers commonly found in processed foods during the digestion process, will help to rationally develop healthier foods, not only capable of promoting satiety but also of reducing hyperlipidaemia and blood cholesterol levels. This will help to address obesity and associated conditions, one of the major challenges currently faced by the EU, . At the host institution (IFR), Cristina Fernandez-Fraguas has used, in particular, a range of colloidal and interfacial methodologies to identify the mechanisms by which food ingredients can influence lipid digestion through either bulk or interfacial effects. She has also carried out experiments of *in vitro* digestion under physiological conditions, following established IFR protocols. The results demonstrate feasible approaches for modifying both the continuous phase and the interface of emulsions by using specific types of food biopolymers. This will allow the design of food matrices able to control bile salt behaviour / transport and hence have a marked impact upon fat digestion and metabolic health.

APPROACH

The project initially looked at the design of emulsion interfaces that hinder adsorption of bile salts as a potential mechanism to reduce adsorption of colipase and lipase and thus to limit the rate of lipolysis. Model emulsions covering a range of responses to digestive conditions were selected; these included systems representative of protein-based ingredients (i.e. β-lactoglobulin - β-lg, α-lactalbumin - α-la, whey protein isolate - WPI), polysaccharide–protein complexes (sugar beet pectin - SBP) and gels (Hydroxypropyl methylcellulose - HPMC). A relevant mixture of two common bile salts (BS), sodium taurocholate (NaTC) and sodium glycodeoxycholate (NaGDC), was used. These studies were then extended to investigate the implication of the BS binding properties of dietary fibre in the control of lipid digestion. Firstly, colloidal methodologies were used in order to characterize the different ingredients and to investigate emulsion stability. A range of interfacial techniques, including particle electrophoresis analysis, tensiometry, interfacial dilatational rheology, interfacial biosensors and AFM, were used to determine changes in interfacial behaviour of the adsorbed layers in presence of BS. The impact of any particularly interesting systems on the extent of lipid digestion was investigated by means of an *in vitro* digestion model under simulated GI conditions.

Strengthening interfacial protein networks using heat-treatment to create digestion resistant-emulsions:

Displacement of protein networks by a model non-ionic surfactant (Tween 20). Heat treatment of β-lg and (α-la+β-lg) networks increased their resistance to displacement by Tween 20.

Resistance of proteins networks to displacement by BS after gastric digestion. Surface potential measurements showed that heat treatment of emulsions made WPI and β-lg networks susceptible to displacement by BS in presence of pepsin. However, in the absence of pepsin, both heated protein networks were resistant to displacement by BS. This phenomenon is possibly due to heat treatment and adsorption increasing the susceptibility of protein networks to pepsinolysis, which then weakens the film, thus predisposing the interface to be more susceptible to adsorption by BS.

Lipolysis of β-lg emulsions. Emulsions appeared to be more susceptible to lipolysis when heat-treated, probably due to changes in the interfacial rheology following gastric conditions as described above.

Comparing the potential of structurally different surface-active polysaccharides (SBP and HPMC) to reduce the extent of lipolysis. Surface tension measurements and interfacial dilatational rheology

showed that both biopolymers were able to compete with BS for the olive oil interface at low BS concentrations. SBP seemed to interact more strongly with BS, probably due to attractive electrostatic interactions between the BS and the protein component of SBP, leading to disruption of the interface at lower BS concentrations than HPMC. The non-ionic nature of HPMC showed a weaker interaction with BS and an interface that was not easily disrupted, exhibiting promise to resist digestion at low BS concentrations. However, SBP- and HPMC-stabilised emulsions presented a similar extent of lipolysis, probably due to the surface being dominated by BS at high BS concentrations.

Investigating SBP-BS interactions using AFM. Force spectroscopy showed that the interfacial structure of oil droplets + SBP clearly became more deformable in the presence of BS, confirming that BS were readily adsorbing into the SBP interface at low BS concentrations.

Implication of the BS binding properties of dietary fibre in the control of lipid digestion:

Cellulose ethers. The potential showed by HPMC to resist digestion at low BS concentrations opened up the question whether it was due to the interfacial properties of the HPMC or binding of BS in solution. Hence, a Dual polarization interferometer (DPI) and a Quartz crystal microbalance (QCMD) were used to explore the formation of mixed cellulose ethers-BS adsorption layers onto a hydrophobic solid-liquid interface by means of sequential and simultaneous adsorption as representative of interfacial and bulk interactions, respectively. Commercial celluloses (HPMC and MC) with different type of substitution, M_w and hydrophobicity were investigated. No substantial differences were found on the overall displacement among cellulose layers formed sequentially based on M_w and substituent nature reflecting that the composition of the interface only has small effect on the displacement by BS. QCMD and DPI mass adsorbed as well as thickness profile curves obtained during simultaneous adsorption of both components, showed that BS interacted with MC and HPMC in a different manner reflecting that the cellulose structure (type and number of substitution) has an impact on the mechanism of interaction between cellulose ethers and BS in the bulk. The different interactions affected the amount of free BS and the functionality of the cellulose/complexes, and consequently how the cellulose is competing for the surface. Specifically, MC was more effective at binding BS in the bulk than HPMC, suggesting weak HPMC-BS interactions and consequently an excess of free BS to compete for the surface in the HPMC system. However, both, low and high M_w MC, followed a similar behaviour reflecting that interaction between MC and BS is not M_w dependent. Mixed cellulose-BS layers formed simultaneously led to greater adsorption than sequentially formed, probably due to adsorption of complexes but at the same time, the adsorbed layers were easier to displace probably because the layer formed by the complexes was weaker.

Impact of β-glucans on the BS binding properties of oat bran: The influence of the structure of individual pure BS, NaTC and NaGDC, on their competitive adsorption with **pure β-glucans** (β-G) was evaluated using interfacial tension (IFT) measurements. A comparison of the simultaneous adsorption of β-G and BS with the adsorption of BS alone shows that β-G promotes the adsorption of BS to the oil interface, supporting the idea of a cooperative adsorption process. Differences in adsorption behaviour between both BS species were observed, corroborating recent results obtained by our group in solid and fluid interfaces. The system NaGDC- β-G readily adsorbed, reflecting absence or very little interaction between components, thus leading to phase separation. In contrast, an interaction between components was observed in the NaTC- β-G system which adsorbed more slowly and showed two competing effects: low concentration of β-G shows cooperative effect but as it increases, the amount of free NaTC is reduced and the adsorption is less pronounced. The presence of salts modified the behaviour and reduced the effect among concentrations in both systems, possibly by screening out the intensity of the interactions between BS and β-G. Additionally, we evaluated the correlation between bile salt binding and **insoluble dietary fibre (IDF),** measuring the adsorption of unbound BS to the oil interface. IFT values confirmed the presence of unbound BS, but their adsorption decreased as the oat bran concentration increased, reflecting that IDF retained or entrapped part of BS. The adsorption of unbound NaTC was much slower than unbound NaGDC, reflecting that the IDF seemed to bind/associate or complex NaTC more effectively than NaGDC. Further experiments using Isothermal Titration Calorimetry are in progress.

CONCLUSIONS: FoodPro ForHealth has successfully enabled the use of colloidal and interfacial tools to investigate the interaction of diverse food structures with digestive enzymes, bio-surfactants and lipids in simulated gastric and duodenal environments. FoodPro ForHealth has shown, firstly, that heat treatment is a successful strategy to enhance the resistance of whey protein interfacial layers to displacement by Tween 20 and bile salts. Secondly, digestion of emulsions stabilised with a naturally occurring protein-polysaccharide complex (SBP) and a non-ionic polymer (HPMC) was similar despite their different competitive adsorption behaviour at low BS concentrations. This reflected that BS are very effective at adsorbing and disrupting interfaces, and they appear to be the critical step for the progression of duodenal

lipolysis. Thirdly, the binding of BS to celluloses in solution can affect displacement by BS in two ways, by changing interfacial properties of the cellulose-BS complexes, and by reducing free BS available to displace cellulose and/or complexes. Dr. Fernandez demonstrated that the potential of cellulose ethers to influence satiety is more related to their ability to sequester BS in solution than their ability to disrupt adsorption of BS at the interface. This important finding, together with the challenge of designing interfaces to restrict adsorption of bile salts, led us to focus our attention on the physic-chemical mechanisms by which a specific dietary fibre, with recognised cholesterol reducing properties, would impact fat digestion. From here, FoodPro ForHealth provided has new insight into how the binding of either soluble (β-Glucans) and insoluble fibres from oats to bile salts in the bulk impact the interfacial properties of the emulsions related to access for digestion. In addition, the different adsorption behaviour observed between different bile salts structures supports the necessity of filling the gaps in our understanding of the roles of various essential components in lipid digestion. FoodPro ForHealth has given us a better, fundamental understanding of how interfacial and bulk properties, solution conditions, and the interactions with bile salts determines the digestibility of food matrices. This is generating renewed scientific interest due to the growing social and economic consequences of the obesity crisis in the developed world. The design of healthier foods to control obesity is a top priority in the EU: it is estimated that treating obesity and related diseases (such as type 2 diabetes and cardio-vascular disorders) takes up to 5% of the EU's total healthcare budget. FoodPro ForHealth has contributed significantly to bridge the knowledge gaps between nutrition and materials science to improve our understanding of how food structure can influence digestion of carbohydrates, proteins and lipids, and how this in turn can influence dietary uptake, satiety, and metabolic health. This knowledge can be exploited in tailoring novel food systems able to control bile salt behaviour / transport as a potential route to modulate lipid absorption. Applying physical and materials science principles to understand fundamental processes, such as bile salt adsorption/interaction, and to modulate digestion and interfacial/bulk properties of complex food structures is a novel, emerging area of industry-relevant research, and may have further health benefits in terms of lipid metabolism and gut health.