Understanding food structure and function in developing food for appetite control

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Abstract

There is an emerging interest in the impact of food structure on digestion behaviour and its relationship to human nutrition. The interactions between individual macronutrients (protein, fat and carbohydrate) in many cases control the rate of digestive processes such as proteolysis and lipolysis. Macronutrient interactions can also control the material properties of ingested foods which can influence post-prandial metabolic responses. This review summarises the current research into how the structure of individual macronutrients impacts digestion and how this relates to appetite.

Key words: protein, carbohydrate, emulsion, satiety, microstructure.

INTRODUCTION

Ongoing public and media interest in food issues such as the obesity epidemic has led to an increased consumer demand for nutritional, high-quality foods with an optimised caloric profile that can be incorporated into a balanced diet. While one of the ongoing concerns of the food industry has always been to produce and provide the consumer with safe food, the nutritional and caloric composition is now becoming equally important. The food industry is currently responding by reformulating their products, especially looking at the salt, sugar and fat content with a particular emphasis on healthier fat compositions. There is also growing evidence that post-prandial behaviours are not governed solely by the relative calorie profile/content of foods, but that additional factors, such as food structure, rheology and breakdown, may also play an important role in the digestive process and subsequent metabolic response.1–4

The interplay between food formulation, process and functional attributes, such as texture, taste, stability, nutrition and satiety, are all related to food microstructure.5,6 The microstructure of food consists of the spatial arrangements of structural elements, such as polymer strands, networks, crystals, droplets and air cells.7 The different structural elements constitute assemblies of biomaterials (proteins, polysaccharides and fat/oils) with different sizes and shapes over a range of different length scales from nanometres (nm) to centimetres (cm). Looking through a microscope at a food product will give intriguing information of the structural elements and their spatial arrangement.8,9 Figure 1 shows a number of micrographs picturing the hierarchical aspects of food structures. Using high-resolution transmission electron microscopy, it is possible to visualise strands of single polymers and assemblies of polymers in supramolecular strands and networks (Figure 1a,b). At the next length scale, structures such as polymer networks and fat globules can be visualised using scanning electron microscopy and confocal laser scanning microscopy (Figures 1c,d). We all experience and enjoy good-tasting food on a daily basis, but what is less understood is the fate of the food after it has been swallowed. This review summarises the current research into how the structure and material properties of proteins, carbohydrates and fats affect digestion and post-prandial metabolic responses.

EFFECT OF PROTEIN MICROSTRUCTURE ON DIGESTION

Protein structure function properties in the digestive system

Of the three primary food groups of protein, fat and carbohydrate, protein is the most effective at providing satiety (on an energy equivalent basis).10 The physiology of protein digestion is a complex affair which is designed to deconstruct proteins into component amino acids which are ultimately absorbed in the gut. The process of proteolysis starts in the stomach through a combination of low pH and the action of the enzyme pepsin, and it continues in the intestine with the action of trypsin, chymotrypsin and carboxypeptidases. In many cases, the efficacy of digestive peptidases is

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dependent on the structure of the protein system in solution, which has a subsequent impact on the kinetics of amino acid absorption. How this process contributes to metabolic behaviours, such as satiety, is of increasing scientific interest and research activity.

Milk proteins as an example

To highlight the importance of a protein’s structure on its digestion, we have chosen to compare the two main protein constituents in milk, that is, casein and the primary whey protein, β-lactoglobulin. It is important to distinguish between micellar casein, which is an assembly of the four primary casein genotypes forming small (50–600 nm) particles of proteins, and caseinate, which consists of a molecular mixture of the four different caseins. In milk, β-lactoglobulin coexists with micellar casein and has a folded globular structure with an extensive secondary structure.

A study by Guo and coworkers compared the proteolysis of these two milk proteins by the digestive enzymes pepsin and trypsin. Their findings showed differences in proteolytic susceptibility depending on the type of protein. The caseinate proteins were more susceptible to proteolysis by pepsin and trypsin compared with β-lactoglobulin. The open structure of the proteins in caseinate allows enzymes greater access to target residues, resulting in rapid proteolysis and faster uptake of amino acids during digestion. In contrast, the folded structure of β-lactoglobulin inhibits enzyme access to potential cleavage sites and, as a consequence, the protein is considerably more resistant to hydrolysis by either pepsin or trypsin.

While the native structure of globular proteins has been shown to be resistant to proteolysis, studies have shown that thermally induced protein unfolding and denaturation result in increasing susceptibility of β-lactoglobulin to pepsin and trypsin proteolysis. It is important to point out that most food manufacturing processes expose the food material to temperatures (e.g. pasteurisation and ultra high temperature), which often leads to unfolding of proteins. Similar to temperature treatments, other food processes, such as high-pressure treatments, have been shown to lead to protein unfolding and exposure of the active sites for the proteases, resulting in enhanced rates of proteolysis.

Molecular interactions

The structural complexity of protein systems is increased further as individual proteins can also interact with each other or other constituents, for example, carbohydrates. Proteins can be cross-linked using enzymes such as transglutaminase, or conjugated with carbohydrates through chemical (Maillard) reactions that occur during cooking and frying. For example, studies of enzymatic cross-linking have shown that for transglutaminase cross-linked casein, there is little impact on proteolysis behaviour arising from the introduction of additional covalent cross-links. This is possibly due to the fact that the cross-linked regions on the protein molecule are not involved in the proteolysis reaction.

The chemical interactions induced by enzymes or the Maillard reaction are irreversible; however, proteins are also susceptible to reversible physicochemical effects such as changes in pH or ionic strength. For example, as the pH of a protein is decreased, it approaches a point where there is a net zero protein charge, the isoelectric point. At the isoelectric point, most proteins will, especially if they are denatured, start to lose solubility and consequently may aggregate and form networks. In the case of milk proteins, there are two important factors that can control the structural changes that occur upon acidification in the stomach. First, the

Figure 1 Micrographs of polymer and food structures. (a) Transmission electron microscopy micrograph of individual locust bean gum molecules. (b) Transmission electron microscopy micrograph of the supramolecular assembly of xanthan. (c) Scanning electron microscopy micrograph of partially coalesced fat droplets in a cream cheese. (d) Confocal laser scanning microscopy micrograph of milk protein (red), locust bean gum (black) and fat (green) in cream cheese.
proteins themselves exhibit buffering capacity which will lead to an increase in stomach pH. This meal-induced change to stomach pH is slowly reversed by the secretion of additional acids into the stomach and by the gradual emptying of the meal from the stomach. The rates of these changes in pH are influencing how the proteins will create structures in the stomach. Second, the resulting structure is also influenced by the contractions of the stomach walls which create shear forces that are affecting how the proteins are interacting.

The effects of pH-dependent protein structuring on gastric behaviour have been reported in a number of studies.\(^{20-22}\) The precipitation of casein around its isoelectric point has been reported to result in delayed gastric emptying while β-lactoglobulin, which is not subject to acid precipitation, empties rapidly into the duodenum. This observed difference in emptying rates has been further related to differences between the two protein systems in terms of overall amino acid uptake, with native β-lactoglobulin amino acids being reportedly taken up at a quicker rate than casein proteins.\(^{22}\)

The above example highlights the complexity of the structural processes occurring during food consumption and the resulting effect on the digestive processes, but it is clear that the state of the protein is influencing these processes and should be taken into account when evaluating post-prandial responses.

**Effect of emulsion microstructure on digestion**

Lipids play an essential, if somewhat controversial, role in the human diet. Lipid emulsions facilitate the delivery and uptake of essential lipophilic nutrients (e.g. vitamins, polyunsaturated fatty acids). Furthermore, the hedonistic sensory properties of lipids are closely associated with the positive perception of the taste and texture of foods.\(^{23}\) However, lipids are also the most energy-dense of the macronutrients, and overconsumption of fat is cited as a leading cause of obesity. The digestion of fat is not only important for the body’s energy economy, but also plays a role in satiety and subsequent energy regulation.\(^{1,10}\) The release of fatty acids in the upper small intestine leads to the secretion of a range of neuropeptides (cholecystokinin and peptide YY) that act to alter gastric emptying and eating behaviour.\(^{24,25}\)

Invariably, all ingested fat ends up as an emulsion either by gastric emulsification or prior to ingestion during the manufacturing process. The structure of these emulsions is determined by the nature of the three phases of the system, the fat phase, the interface and the aqueous phase.\(^{26}\) Emulsion size is readily controlled by astute selection of emulsifier and homogenisation conditions, with sizes ranging from 100 nm to 100 μm being readily achievable. It is important to appreciate that as the droplet size is decreased, the emulsion’s surface area is increased. The structures of the most common kinds of food emulsion states are depicted in Figure 2. The oil/fat droplets can be designed to be homogeneously dispersed (Figure 2a) or to flocculate (Figure 2b), effectively it is possible to convert a high-surface-area emulsion into a low-surface-area aggregate. Alternatively, partial coalescence can be induced leading to the formation of more persistent structures with larger ‘lumps of fat’ (Figure 2c). If the emulsion is destabilised completely, the oil phase will separate forming a layer on top of the aqueous phase (Figure 2d).

**Lipolysis of emulsions**

The digestion of fats involves the enzymatic conversion of triglycerides into fatty acids and 2-monoglyceride. Fat digestion occurs at the surface of insoluble fat droplets via the action of digestive lipases.\(^{27,28}\) Lipolysis is initiated in the stomach by the action of acid-stable gastric lipase and is continued in the duodenum by the dual action of gastric lipase and colipase-dependent pancreatic lipase in the presence of surface active bile salts.\(^{27,28}\) High amounts of pancreatic lipase are secreted during the consumption of a meal (200–250 mg), and these levels are normally more than enough to digest all of the ingested fat.\(^{29-31}\)

The structure of the emulsion has a considerable impact on lipolysis, with emulsion surface area being the key physicochemical factor affecting fat digestion. Figure 3 shows the effect of emulsion size on in vitro lipolysis rate. The lipolysis rate scales with emulsion size, the emulsion with an average droplet size of 160 nm having the highest interfacial area and undergoing the fastest lipolysis. The effect emulsion structure has on digestion has also been demonstrated by several in vivo studies.\(^{32,33}\) The work by Armand and coworkers,\(^{33}\) in a study of gastric aspirates, demonstrated that the gastric lipolysis of a fine 0.7 ± 0.2-μm emulsion was almost three times more than a 10.1 ± 0.9-μm coarse emulsion. Similar behaviour was observed in the duodenum where the fine emulsion underwent almost twice the lipolysis of the coarse emulsion. The effect emulsion structure has on fat absorption/uptake can be understood by monitoring blood plasma triglyceride concentration (Figure 4). A comparison of the digestion and uptake of free versus emulsified oil reveals that emulsification leads to a much earlier and larger peak in blood plasma triglycerides, indicating more efficient digestion and uptake.\(^{32}\)
The impact emulsion structure has on the lipolysis rate can be understood from the fact that, under physiological conditions, the pancreas produces an excess of lipase relative to the amount of ingested fat. Therefore, the rate of lipolysis is controlled, not by the amount of enzyme, but by the amount of interfacial area available for lipase binding. Furthermore, the action of gastric lipase is inhibited by lipolysis end products. The accumulation of fatty acids at the interface results in the formation of insoluble aggregates that entrap gastric lipase, inactivating the enzyme. Increasing fat droplet interfacial area means that gastric lipolysis can progress further before the fatty acid concentration is sufficient to cause inhibition.

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A growing understanding of the interaction of emulsions with gastrointestinal environments is enabling researchers to design emulsions with specific structures in vivo. An example is the work by Wickham and coworkers who exploited the different acid stabilities of two surfactants to structure the emulsions in vivo. The two emulsions had the same initial particle size (average diameter ~3.6 μm), but acidification in the stomach lead to different gastric microstructures. One emulsion was acid-stable and retained its original structure in the stomach. The other emulsion separated into two phases upon acidification, which was verified using magnetic resonance imaging (MRI). In Figure 5, it can be seen that the deliberate gastric structuring of these emulsions altered the rate of stomach emptying. The homogeneous emulsion had a constant stomach emptying rate over 3.5 hours. The destabilised emulsion initially underwent rapid emptying of the aqueous phase followed by slow emptying of the oil layer floating on top of the water phase. In addition to modulating the emptying rate of the stomach, the differences in fat digestion also affected the rate of cholecystokinin release. For both responses, this has been shown to affect a satiety response in human subjects. It is clear that emulsions can be deliberately designed to have contrasting structural behaviours in vivo and that these behaviours are modulating lipolysis rate, hormone response and gastric emptying.

**Effect of high-molecular-weight carbohydrate structures on gastric transit**

Carbohydrates are the third macronutrient group of nutritional importance. Of increasing interest is the role that
high-molecular-weight carbohydrates, or polysaccharides play during digestion. Polysaccharides are often referred to as dietary fibres and have been reported as modulating satiety responses. The polysaccharides commonly present in food products are often divided into starch and non-starch polysaccharides, based on their digestibility in the gastrointestinal tract. Starch-based polysaccharides are converted to glucose by α-amylase, a digestive enzyme present in saliva and the small intestine. Non-starch polysaccharides (NSP), such as cellulose, pectin, galactomannans, glucans, are resistant to α-amylase digestion but can undergo fermentation in the colon. The NSP can be subdivided into two groups depending on their solubility. This division is ambiguous as it depends on what solvent they are dissolved in. However, with the exception of cellulose, most of these NSP are soluble in the conditions they would experience in foods or during food manufacture. In the manufacturing industry or indeed when you are cooking at home, the unique structuring properties of these polysaccharides are often taken advantage of to create texture and to thicken the food material. In essence, the mechanical properties of the food material are governed by the assembly of the different constituents into higher-order structures. The chemistry and physics of these processes as a function of polymer parameters (configuration, concentration, molecular weight and distribution) and physiochemical conditions (pH, salt, temperature, pressure) have been extensively researched (for reviews see Glicksman, and Whistler and BeMiller).

**In-body functionality of high-molecular-weight carbohydrates**

NSP plays an important role in our diet and has been reported to slow down small intestine transit, lower blood plasma cholesterol and improve bowel function. For appetite control, the primary function for the NSP is to modulate the rate of stomach emptying, with a prolonged residence time of the food in the stomach leading to a feeling of fullness and less hunger. The NSP can contribute to this through increasing the viscosity or being in a gelled state. It is important to distinguish and also understand the difference between these two solution states of NSP. In a viscous solution, the polymers are interacting with each other because of entanglements, that is, there are no permanent bonds between individual polymers, and it is possible to decrease the viscosity through dilution. Dilution will lead to a disentanglement of the polymers resulting in a decrease in viscosity. While in the case of polysaccharide gels (networks), the polymers are cross-linked into a 3D structure and the addition of solvent will, in most cases, not change the integrity of the network. Many studies have examined the effect of stomach content viscosity on stomach emptying and satiety. Marciani and coworkers used MRI to visualise and study the dilution and stomach emptying of meals with varying locust bean gum content. They showed that the viscous fluid meal was slowly diluted by gastric and salivary secretion and that the higher-viscosity meal was emptied at a slower rate. The subjects in the study also reported feeling an increased sense of fullness. In addition to modulating gastric transit, viscous fluids are also highly efficient in slowing down diffusion of enzymes and nutrients, which affects the rate of nutrient release and digestive processes.

With an understanding of the solution and gelation behaviour of NSP, scientists have recently been able to design and test fluid meals which are forming gel lumps when exposed to gastric conditions. Hoad and coworkers used alginate with varying mannuronic acid and guluronic acid content to form gels of different strength under gastric acid conditions. Their results suggest that the formation of gel lumps of sufficient gel strength slowed down gastric emptying, leading to an increased feeling of fullness. They suggested that the formation of gel lumps caused antral distension and activated tension receptors in the stomach walls leading to slower transit.

When designing gastric gelling systems, it is important to understand that even within a particular class of polysaccharides, there may be differences in molecular structure of the polysaccharide, for example, degree of esterification of pectins or mannuronic : guluronic ratios in alginates. These molecular differences will influence solution and network-forming behaviour of the polymer and also their response to different environments (pH, salt conditions and temperature). In summary, understanding how polysaccharides can form different microstructures makes it possible to control the mechanical properties of ingested foods and associated satiety responses.

**CONCLUSIONS**

The review summarises the evidence that suggests it is not only the gross macronutrient composition of food that influences the gastrointestinal physiological responses, but also how these macromolecules are assembled into different structures during the food manufacture process and gastric transit. The interaction of food microstructures with the enzymatic and mechanical breakdown process of digestion is not well understood. Cross-disciplinary research, bridging food structuring/manufacturing and gastrointestinal physiology research, in conjunction with the development of non-invasive measurement techniques, will support the development of future foods with a designed functional behaviour in the body. Such foods may have the potential to combat weight and obesity issues.

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**CONFLICT OF INTEREST**

No conflict of interest has been declared by L. Lundin, M. Golding or T.J. Wooster.
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