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Author Comments:	Apologies - I have now annotated the selected priority references. I have also attached a CORRECTED FINAL version of the manuscript

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Abstract

We investigate recent advances in the ~~major~~ Chemical Engineering aspects of food structuring agents and macronutrients: carbohydrates, proteins and lipids, and also the fate of food upon ingestion. Prebiotic effects on host-microbe interactions enable improved immune response and pathogen control. Formulation Engineering is an emerging area in the field of Chemical Engineering and requires detailed knowledge of the materials used, and the processes by which they are transformed / functionalised into products. Understanding 'comb-like' polymers and their interactions provide new structuring opportunities. Sustainable sourcing of alternative protein sources, natural lipid organelles and structuring of liquid oils are framed in a waste valorisation approach, utilising biotechnological approaches for new functionalities. Controlling natural and fabricated microstructures enable controlled digestion profiles.

1. Introduction

While modern genetic techniques are employed to change the properties of food materials at the agricultural (pre-farm gate) level [1], here we will focus on the developments pertinent to Chemical Engineering of food ingredients and foods at the processing stage, recent developments of prebiotic effects and the design and testing of foods for controlled digestion and delivery of healthy excipients, address the consumer need for minimally processed foods and explore food waste valorisation.

2. Carbohydrates

Dietary fibres continue to be a focus of ongoing research, from the extraction and isolation of specific structures that are reported to have prebiotic effects, to the elucidation of functionality of natural microstructures where the effects of soluble and insoluble fractions are examined [2, 3, 4], leading to formulation, processing and design rules for foods which have optimised functionality.

2.1. Prebiotics

Prebiotics are considered to be dietary substances that are selectively utilised by host micro-organisms to confer benefits to health [5], being non-digestible food ingredients that stimulate beneficial bacterial growth in the colon and are widely used as nutritional supplements in human and animal diets to promote gut health. Maintaining gut health is essential for human wellbeing, and the welfare and productivity of animals [6]. In addition to nutrient digestion and absorption, the intestinal mucosa constitutes a physical and immunological protective barrier for the integrity of the intestinal tract, however, systematic information regarding the interaction between prebiotic diet, the structure of gut microbiota and the host gene expression is limited.

Recent reports demonstrate that galacto-oligosaccharides (GOS) can improve the performance of farm animals by promoting profound differences in desirable bacterial groups inhabiting the gut [7]. In broiler chickens GOS has been demonstrated to select key species of autochthonous *Lactobacillus* that promote bird performance [8], improving zootechnical performance and concomitantly stimulated specific increases in expression of the pro-inflammatory cytokine IL-17A in ileal and cecal tissues. Shifts in the cecal bacterial communities primed the intestinal innate immunity of birds in the absence of pathogen challenge. Combinations of prebiotics with beneficial bacteria (synbiotics) that can engage with host innate immunity have the potential to resist or reduce zoonotic pathogens [9, 10]. Such knowledge will promote Ffuture studies will to design and establish dietary interventions to and bring about the changes in the intestinal microbiota and immune response that target endemic pathogen species.

2.2. *Comb-like polysaccharides: new opportunities for targeted gelation and film-forming properties.*

The mucilage polysaccharides from several species e.g. *Plantago*, are densely decorated by side chains with degree of branching higher than 90%, which classifies them as highly branched polymers (Figure 1). Most mucilage polysaccharides are comb-like polymers and adopt a highly swollen coil-like conformation similar to linear polymers in dilute solution.

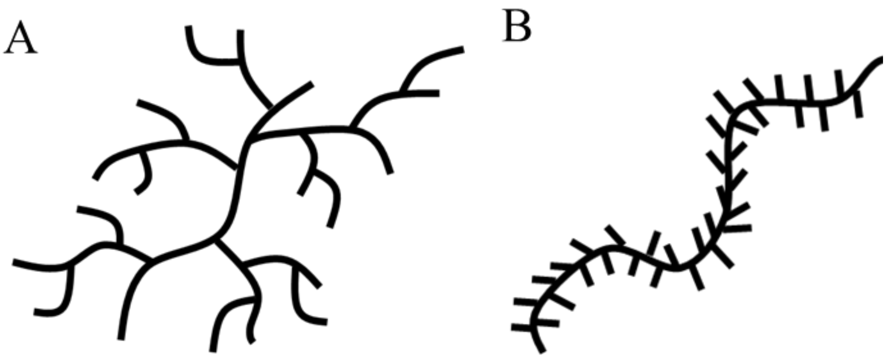

Figure 1 A scheme of hyper-branched polymer (a) and comb-like polymer (b).

The properties of side chains have a great impact on molecular conformation and interactions. Weak interactions between side chains lead to preferably perpendicular orientation of side chain to the backbone, but under the strong interaction, all side chains become strongly tilted to the backbone. Therefore, the comb-like polymers tend to show higher rigidity with same length of side chain under strong attraction than that under weak attraction. If the side chains of polymers are chemically different from each other, the microphase separation of side chains within a single comb-like polymer occur in the solvent that is ‘good’ solvent for one side chain component but ‘bad’ solvent for another one [11]. Therefore, the same cylindrical brush polymer will adopt diverse architecture through changing the solvent quality and provide new engineering design rules for the use of such emerging and under-utilised materials.

The presence of side chain decorations has also been discussed in the comb-like polymers’ interaction with cellulose [12, 13] and their interaction with other rigid polymers such as xanthan [14]. These developments open up the future possibilities for the functional design of composites of natural origin and is a hotbed of current and future research. Recently some of these principles have been employed in creating 3D printed structures [15] where the key process steps are married to the control of molecular interactions as a function of moisture content and thermal profile.

3. Proteins

Animal proteins from eggs and milk are typically used as functional ingredients creating emulsion, foam and gel microstructures in food products, as well as providing essential nutrition, however their production causes a high environmental stress. Plant based proteins are an alternative source, with functionality of soy and wheat proteins extensively studied [16], however changing consumer demands has furthered the search for even more alternatives sources such as yellow pea, chickpea, lentil and faba bean proteins, all of which have been shown to have comparable functionality to animal proteins [17, 18].

The use of insect protein, with the nutritional value successfully demonstrated in fish feeding trials with up to 50% of fish meal replaced with black soldier fly without any adverse effects [19], as well as the low environmental stress of insect breeding, presents insects as another valuable source of protein. Functional properties of insect proteins have also been found to include interfacial properties with mealworm larvae protein isolate, at a lower concentration, having comparable emulsification functionality to whey protein [20], with similar studies noting gelation and foaming properties of protein isolates from a range of insect species. Therefore the use of environmentality friendly and sustainable source of protein are emerging to offset the environmental burden of the use of conventional materials.

While there have been a significant amount of studies on the potential to replace animal proteins with these more sustainably sourced proteins, little attention has been paid to the sustainability of the process to manufacture these functional protein ingredients. For example, the processing of legume flour to a protein isolate can require 80kg water, 22kg hexane, 40g NaOH, 40g HCl and 30MJ energy and result in one kg of legume flour processed with the creation of 30 wt% of the initial material as a by-product [21, 22]; a by-product rich in lipid and carbohydrate.

Schutyser et al. [22] have however analysed the potential to use a combination of milling and air classification to obtain protein-enriched fractions (49-70% protein) without further purification, and therefore a lower energy was used to produce an ingredient which retained better solubility than conventional protein isolates. Similarly, a milling and sieving process was used to produce a pea flour (20% protein) which had comparable interfacial behaviour to a pea protein concentrate (58% protein), showing that the presence of non-protein materials, such as starch granules, did not impact functionality [23]. In addition to these separation processes, eco-innovative technologies such as electrostatic separation, microwave-, ultrasound-, pulsed electric energy and high pressure-assisted extraction are also being investigated [24]. The inclusion of a minimally processed sustainable protein ingredient into a food product will be the next challenge required to ensure a sustainable protein supply, with consideration for the beneficial nutritional factors these ingredients could provide as well as quantifying the anti-nutritional components which may be retained in the minimally processed ingredient.

4. Lipids

Lipids are essential components of most foods: in addition to their high energy value, they affect food texture, carry aromas and provide essential nutrients. Oils and fats are composed of triacylglycerol (TAG) molecules (‘simple’ lipids). These make up most of the lipids we consume, whereas, ‘Complex’ lipids are the building blocks of the membranes and organelles of cells in the food that we eat. They can be extracted for functional applications in foods: for example lecithin (phosphatidylcholine), one of the most abundant membrane lipids in the biosphere, is used widely in the food industry as an emulsifier.

Oil is extracted from oilseeds or soybeans using organic solvents, however, instead of destroying cellular structures there is merit in recovering intact organelles. Devising simple processes to recover lipid-rich organelles intact, establish their physico-chemical properties *ex-vivo*, and probe how they are digested in the gastro-intestinal tract are topics with future potential. Examples of such organelles are now discussed here.

4.1. <u>Oil Bodies</u>

Oil Bodies (Oleosomes) are micron-sized organelles that store oil in desiccant-resistant seeds. The oil-core is covered by a layer of complex lipids (phospholipids) studded with proteins (mostly oleosin). Figure 2 shows images of oil bodies and a schematic, the latter is stylised to clarify the structure of the surface constituents; in reality there are approximately $8x10^5$ oleosin molecules and $107x10^5$ phospholipid molecules per oil body.

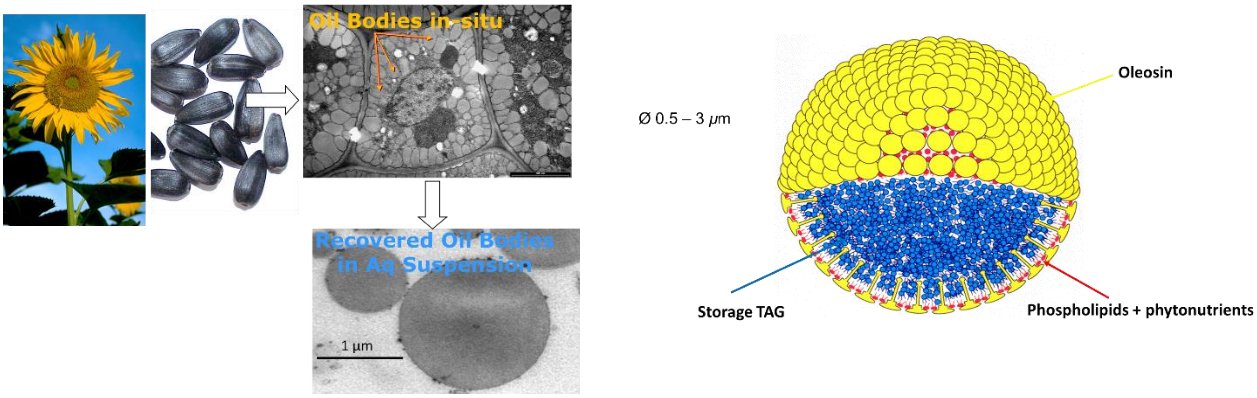

Figure 2 Electron micrographs of oil bodies in-situ and ex-vivo and a schematic of their structure.

Oil bodies are natural lipid droplets with a host of properties that make them suitable as functional ingredients in food and personal care/pharmaceutical applications. They are also appealing in terms of their sustainability: oil bodies are recovered using water (not hazardous organic solvents) and can be dispersed directly in water, thus avoiding the need to re-emulsify the oil [25, 26, 27]. Oleosins are also being used as novel emulsifiers [28].

4.2. <u>Chloroplasts</u>

Chloroplasts are organelles that convert sunlight energy to chemical energy via the process of photosynthesis. Given their ubiquity in the biosphere, they contain the planet's most abundant lipids. Stacks of internal multicomponent membranes (thylakoids) (Figure 3) are composed of complex lipids, galactolipid being the main one, studded with an array of proteins.

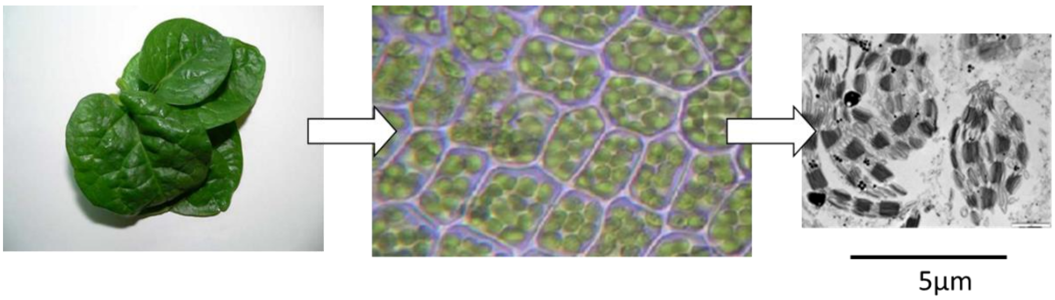

Figure 3 Location and Morphology of Chloroplasts

A similar framework of research questions to study chloroplasts can be used as for the study of oil bodies. Underutilised biomass such as postharvest pea vine field residue (haulm or pea straw) is rich in chloroplasts which can be recovered by a juicing process and concentrated by centrifugation or spray-drying. Just a few grams of this chloroplast-rich fraction can, in theory, supply most of a human adult's vitamin A, E, K_1, Manganese and Iron requirements; it also contains significant levels of omega-3 fatty acids and health beneficial carotenoids [29, 30]. Chloroplasts have been used for both emulsion stabilisation [31, 32] and appetite suppression [33], indicating that their use in foods as ingredients is gaining momentum.

4.3. Oleogels

While three models have been proposed to describe the liquid-state ordering in melted TAGs (the paralamellar (smectic) liquid crystal, the nematic liquid crystal, and the discotic model), no conclusive answers have been provided to describe the structure of molten TAGs nor the first stages of nucleation and crystal growth.

Given that natural oils and fats are complex mixtures of triglycerides (TAGs), di- and mono-glycerides, free fatty acids and some other minor components such as wax esters, none of the existing models takes into account the role played by the minor components. Furthermore, the molecular phenomena preceding fat crystallisation and the molecular patterns at this stage remain unclear and are open for discussion.

Recent work carried out on the pre-nucleation stages of cocoa butter using small-angle X-ray scattering analysis suggests that TAGs self-assemble into lamellar clusters within the melt [34] (Figure 4).

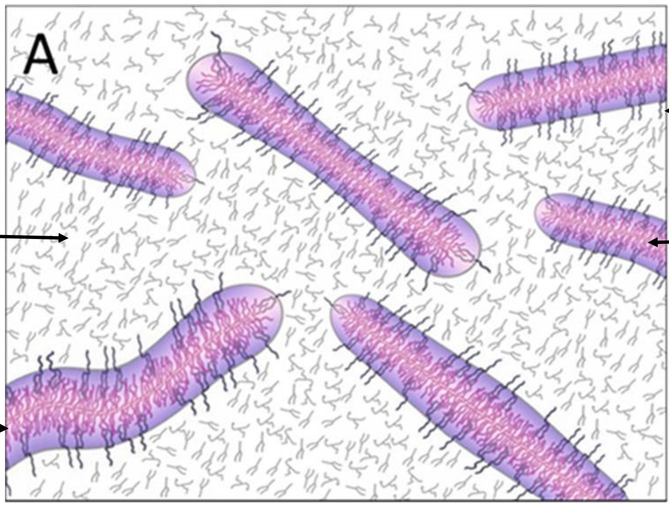

Figure 4: Schematic sectional representation of the lamellar TAG assembly model. Schematic taken from [34].

Plant waxes, such as rice bran wax (RBW), have been identified as powerful oleogelators: heated to temperatures above the final melting point and upon cooling form a solid 3D network entrapping the liquid oil [35]. The relative properties of oleogelation are dependent upon the type of wax/oil used, the cooling rate and quench depth, and the use of shear during gelation [36]. Following from the discussion on pre-nucleation of molten TAGs the physical state produced when the wax-oil mixture is heated above the final melting point of the wax to produce a melt can be questioned as to whether it behaves as a homogeneous isotropic liquid. Complex molecular interactions may occur in the melts, and that a “quasi-smectic” may be formed by wax-esters determining the measured oleogel physical properties and may be affected in wax blends [37] and the effect of other nutritional materials [38].

5. Digestion

Due to the complex, multidisciplinary nature of digestive processes, design of methods is challenging and highly depends on the scope of the study [39]. Typically, it involves *in-vivo*, *ex-vivo*, *in-vitro*, *in-silico* features, or their combinations. A plethora of static and dynamic *in-vitro* models have been developed that are partially physiological relevant [40]. Despite significant progress, the ideal holistic, high throughput, affordable, user-friendly *in-vitro* methodology that would address challenges such as understanding interlinked phenomena at different length scales and will substantially reduce the need of costly, difficult *in-vivo* experiments is yet to be established.

In recent years, structuring of foods and delivery of clinically relevant drugs for controlled digestion has gained increased interest [41]. For example, the high molecular weight and hydrophilicity of proteins impede intestinal absorption. Thus, therapeutic proteins are commonly administered by parenteral route, with insulin, for instance, being administered subcutaneously in the treatment of diabetes mellitus.

However, oral administration is regarded as a better, cost-effective and acceptable route of administration that circumvents the use of needles and other injection materials [42]. Therefore, several attempts have been made to develop oral carriers able to deliver insulin in a continuous and efficient manner, avoiding local pain, immune reactions and potential contamination. Furthermore, oral administration of insulin better mimics the normal insulin pathway in the body after endogenous secretion, providing improved glucose homeostasis.

Insight has been provided into the principles outlining the design of foods for controlled digestion and health active delivery [43]. Indeed the development of an understanding on how natural structures deliver their payload, as a result of the plant cell wall architecture and robustness have been highlighted for e.g. lipid digestion [29]. However, with knowledge of physical stability of microstructures Spyropoulos et al. [44] have developed the ability for multi-active deliver from simple O/W emulsions.

Development of in-depth understanding of digestion and prediction of digestibility of different foods and clinically relevant therapeutic drugs is therefore relevant and open to improvements.

6. Food Waste Valorisation

Ayala-Zavala et el. [45], Tokusoglu [46] and Gedi et al. [47] highlight the current opportunities in the use of by-products. Effects of hydrothermal processing of waste cocoa and coffee grounds in relocating lignin to provide natural Pickering particles able to stabilise both W/O and O/W emulsions provide real novelty in this area [48]. Sridharan et al. [23] has also recently indicated that the need for high levels of refining of proteins is not necessary for the creation of food emulsions, and Ren et al. [13] has shown the applicability of mechanically processing cellulose. Enzymatic approaches have also been used to functionalise natural materials for use as ingredients directly, or for increasing processability in food manufacturing [49] and improving product quality and decreasing nutritional losses in an eco-friendly manner [50].

7. Conclusion

Novel views of more natural and environmentally friendly approaches to creating structure with carbohydrates, proteins and lipids, aligned to the utilisation of was once viewed as ‘waste’ have opened new avenues of research. With the developments of a greater focus on the fate of food structures during digestion, and the ability to be able to parameterise digestion profiles opens up new directions for prediction, and the opportunity to begin to formulate for validated functionality.

Exciting times are ahead in this area of Food Biotechnology.

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Response to reviewer:

The reviewer commented that the article needed to be more weighted towards Chemical Engineering.

Response: while the original article was written with a Chemical Engineering audience in mind – the revised version includes more of a steer towards Chemical Engineering – specifically the emerging area of ‘Formulation Engineering’.

AUTHOR DECLARATION TEMPLATE

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (tim.foster@nottingham.ac.uk)

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