Engineering strategies to modulate nutrient digestion kinetics and bioaccessibility of plant-based foods

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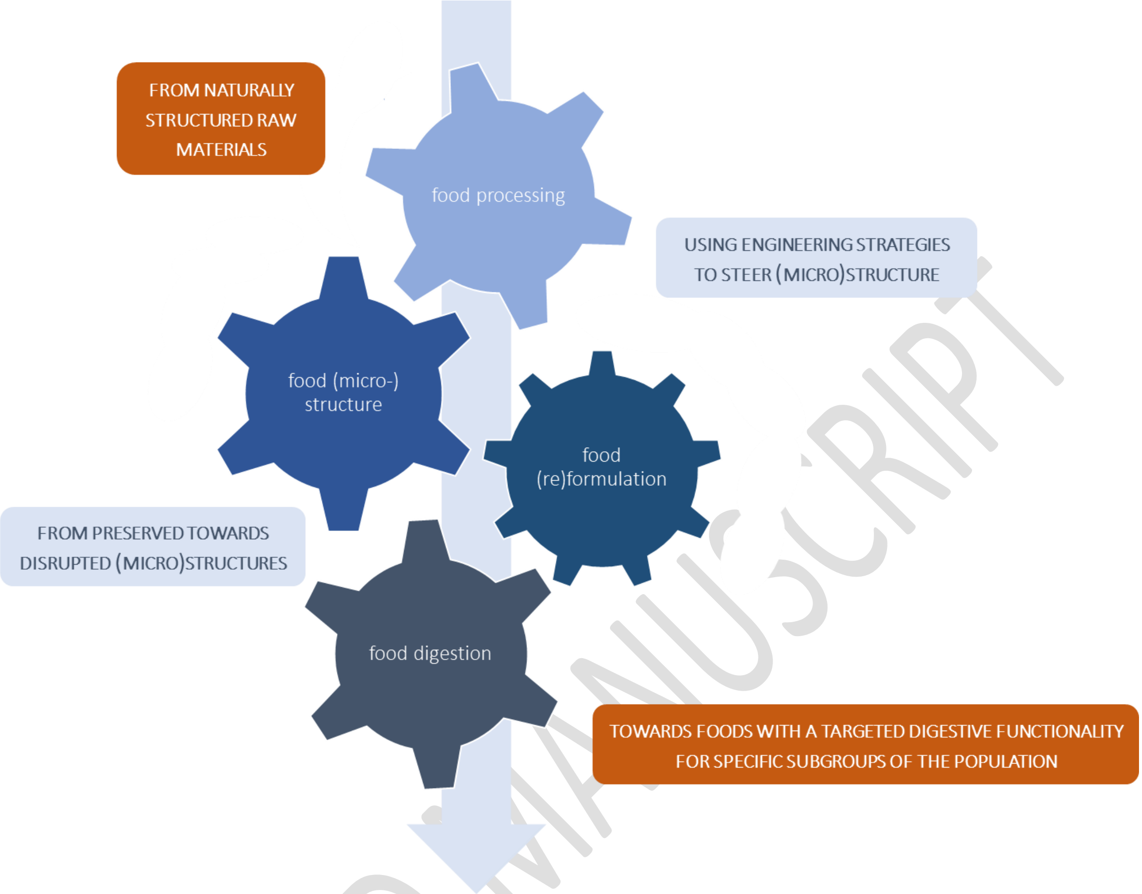
Abstract

Since plant-based raw materials present a relative high level of nutrient encapsulation, processing is often essential to increase susceptibility of nutrients to digestive compounds or to release nutrients from their natural entrapment to become available for digestion/absorption. Next to food processing, food (re)formulation is also an valuable strategy to steer nutrient digestive functionalities. These two food design strategies create a range of different food (micro)structures which are broken down along the gastrointestinal tract determining nutrients digestibility/bioaccessibility. Additionally, the role of digestion conditions on nutrient digestion/bioaccessibility cannot be ignored, paving the way for engineering strategies to develop food for specific target populations.

Keywords: processing; food design; microstructure; food formulation; pulses

Graphical abstract



1 Introduction

Food has a complex organization [1] as it is structured by different digestible and non-digestible compounds. Food composition determines the maximum amount of metabolites and bioactives that can become available for absorption and metabolism [2]. However, food is not a sum of nutrients, yet diverse food structural properties and food formulations result in different digestion/bioaccessibility kinetics and physiological responses.

Food processing includes a variety of unit operations, the so-called food processing chain, to transform raw or fresh foods into edible food products with increased shelf life and food safety [3,4]. This chain is including but not limited to washing, reducing particle size (e.g. cutting, mixing), extracting, dehydrating, heating (e.g. blanching, pasteurizing, cooking), refrigerating, freezing, or a combination of these processes [3]. Although each step of the processing chain has its specific impact on the quality of a food product, the accumulated impact determines the final food quality.

Food quality is a multi-dimensional concept involving objective and subjective dimensions. Nutritional quality is one aspect which is often judged based on the composition of a food [5]. As a result, foods have predominantly been categorized based on nutrient composition. However, food classifications based on nutritional composition solely fail to include nutrient release patterns, which significantly affect physiological responses. Therefore, such classifications do not give a complete image of the nutritional quality of the food, hereby insufficiently informing consumers. Therefore, in the last decades, new types of classifications were on the rise, such as the NOVA classification, based on the extent and purpose of processing. The NOVA classification groups foods into four categories from unprocessed over (minimally) processed foods or ingredients to ultra-processed food products [6]. Especially ultra-processed foods gained much attention, also from consumers, as they are often linked with negative health effects [7]. In practice, ultra-processed foods are identified based on the number of ingredients they contain and specifically the presence of one or multiple ingredients not used in a regular kitchen [6]. It should be noted however, that it is not processing itself that causes food products to be unhealthy, but rather the combination of ingredients, created or isolated through processing, into a food product. Those ultra-processed foods are frequently made from individual, disintegrated ingredients so the final product has no or little natural structure, resulting in a (too) fast digestion. In other words, the (micro)structural organization of nutrients within a food product (and the lack thereof in ultra-processed foods) is most probably a determining factor in foods' nutritional functionality and consequently health effects. Hence, researchers shifted from studying food composition towards studying food structural properties and their influence on nutrient digestion or bioaccessibility [2,8]. These structural properties are frequently engineered by targeted food design (e.g. processing and/or formulation) to steer the nutrient functionality.

This review will therefore give a brief overview of recent engineering approaches to steer food (micro-)structure and in turn nutrient digestion kinetics and/or bioaccessibility. Specific attention will be given to the cases of food processing and food (re)formulation as strategies to regulate food microstructure and in turn nutrient release, digestibility and/or bioaccessibility. The plant-based food matrices mainly discussed are pulses as those require processing before consumption. In these cases, appropriate processing (sequences) help to preserve natural microstructural properties attenuating digestion. Additionally, specific fruits and vegetables are briefly discussed when relevant as well as oil-in-water emulsions.

2 Food processing to steer nutrient *in vitro* digestion or bioaccessibility

2.1 Food processing as a tool to create edible foods

In the case of pulses (i.e. annual leguminous crops harvested solely for use as dry grains), processing is essential to provide a consumable product that has low levels of potentially toxic antinutritional factors and so is more susceptible to digestion [9,10]. A first processing step applied in this case is soaking, to hydrate the dry pulse seed. Generally, water is used as the soaking medium, yet cations can be added to steer subsequent cooking time. Soaking time and temperature can be varied as well which also impact cooking time [11]. Soaked pulse seeds are mostly thermally treated to create palatable pulse seeds. The heat applied induces several structural modifications within a pulse seed like cell wall, starch, and protein changes [9]. The cumulative impact of the intrinsic properties of a pulse seed and extrinsic processing conditions will determine the final hardness of a pulse seed which is often linked to digestion properties [9,11]. Only sufficiently softened pulse seeds are consumed.

In the case of fruits and vegetables, processing is generally not essential to create consumable products. However, especially in the case of vegetables, processing is frequently applied to increase shelf life and/or create a more pleasant and digestible product. The application of processing to fruits and vegetables can have both negative and positive effects on particular nutrients [12]. In this sense, it is known that certain phytochemicals such as glucosinolates and S-alk(en)yl-L-cysteine sulfoxides, are precursors of bioactive compounds, but do not affect health in their original state. Conversion of these non-bioactive precursors only takes place when endogenous enzymes interact with their substrate which can be initiated through processing (e.g. particle size reduction techniques) [13,14]. In contrast, other health-related compounds such as vitamins and carotenoids are sensitive to light, oxygen, and/or heat and as a result, processing often negatively impacts their concentration [12,15]. The nutrient concentration present after creating such edible food products is one aspect that determines the amount of metabolites and bioactives that have potential to be absorbed by the body (i.e. bioaccessible fraction).

2.2 Food processing as a tool to alter microstructure of foods

The term microstructure is frequently used to refer to elements present and interactions occurring among them at the microscopic level (below the 100 µm range). Typical examples are plant cells, cell walls, starch granules, protein assemblies, oil droplets and colloidal structures [16]. It became clear that food microstructure plays a controlling role in the digestive fate of nutrients since different food microstructures are broken down to different extents along the gastrointestinal tract [17].

Pulses are an excellent case study on this matter. They consist of a seed coat surrounding two cotyledons. Within a cotyledon, numerous cells are present containing starch granules and protein bodies, with each cell being surrounded by a cell wall [9]. This complex structural organization of pulse seeds results in a natural encapsulation of nutrients, which can be strategically modified by processing [18]. It this sense, different foods and ingredients can be created from pulses with different (digestive) functionalities.

Pulses are mostly consumed as seeds after processing. Generally, longer cooking times are linked to a higher level of cell separation due to pectin solubilization in the middle lamella and therefore higher proportions of individual cotyledon cells upon mechanical disintegration [19]. Oppositely, insufficient cooking, gives rise to cell breakage and the release of free macronutrients. In other pulse types, shorter cooking times have been related to the separation of cell clusters. These differences in microscopical organization give rise to different levels of nutrient encapsulation and thus (starch) digestion kinetics [20][19,21]. Additionally, Edwards et al. [22] investigated the effect of cell wall properties of two seed producing plants, durum wheat (monocot) *versus* chickpea (dicot), and the consequences on *in vitro* starch digestion kinetics. These authors concluded that intrinsic differences in cell wall properties of pulses (cotyledon) and wheat (endosperm) determined the rate and extent of starch digestion, with pulse cell walls being less permeable for α-amylase [19]. Different types of processing have been explored for pulses as well [22,23], yet hydrothermal processing remains the most applied way of pulse seed softening. Overall, the delayed starch digestion kinetics associated with encapsulated starch granules in pulse seeds gives large potential in the formulation of low glycemic index foods (cfr. Section 3) in which ingredients derived from pulse seeds play an innovative role.

In recent years, research not only focused on understanding starch digestion as affected by the primary cell wall barrier of pulses, yet the protein matrix entrapping starch granules and therefore acting as a secondary barrier got in the spotlight as well. More specifically, gradual proteolysis and reduction of this secondary barrier, leading to amylolysis facilitation is being investigated, for example by Do et al. [24] for navy beans. Although there are several indications that this hypothesis holds for all pulses, it should still be confirmed for understudied pulse types. In this context, new approaches and quantification methods were developed to gain more in-depth insight into protein digestion of pulses [25] but also of other plant-based foods [26,27].

Generally, it can be stated that hydrothermal processing of pulses is associated with improved protein nutritional quality. This is explained by thermal degradation of protease inhibitors and causing protein denaturation reducing protease resistance. Oppositely, the Maillard reaction and/or protein denaturation increasing protease resistance is linked to thermal processes in absence of moisture or when overheating protein [10]. Improving the protein nutritional quality of pulse-based products continues to be a challenge in which targeted processing techniques can play a key role.

Fruits and vegetables also possess naturally encapsulated nutrients, which are often localized within specific plant cell structures surrounded by an undigestible cell wall. Consequently, cell wall intactness is a determining factor in the bioaccessibility of these nutrients. Processing can be employed to stimulate nutrient release and/or bioaccessibility, by affecting cell wall integrity and/or other microstructural properties. Different processing techniques can be utilized for this aim: thermal processing, particle size reduction by high pressure homogenization or mixing, pulsed electric field treatments, enzymatic processes, etc. [28]. These processes can be applied for water-soluble nutrients which are naturally entrapped in cell structures to promote their release and/or absorption [14,29]. However, for lipid-soluble nutrients such as carotenoids and vitamin D, the nutrient amount that is released from the matrix is not necessarily equal to the amount that is absorbed. In these cases, the (lipid) digestion process during which mixed micelles are formed as well as nutrient interactions play a key role in the nutrient fraction finally absorbed [18,30].

3 Food formulation to control nutrient digestion kinetics

Not only food processing but also food (re)formulation can be applied as a strategy to steer nutrient digestibility (kinetics) or bioaccessibility, and is very often done by strategically including nutrients with a specific microstructural organization.

In the case of oil-in-water emulsions for example, it became obvious that several factors influence lipid digestion: (i) oil phase properties, (ii) oil droplet size, (iii) interfacial properties, (iv) properties of the continuous phase [31]. However, one recent research trend aims to explore and understand the use of a mix of emulsifiers to modulate emulsion stability and/or lipid digestion [32,33]. For this, both traditional molecular emulsifiers are studied as well as colloidal (nano)particles such as proteins, polysaccharides and polyphenols. Pickering and nano-emulsions are of interest in food industry to act as delivery systems for bioactives (e.g. curcumin, carotenoids), replacers of fats and yolk, or edible food packaging films [34]. Furthermore, natural oil droplets and emulsions extracted from different sources like nuts and oil seeds, gained more attention from researchers to replace synthetic oils (droplets) in a range (food) products [35,36].

Another emerging trend is to reformulate current food products, driven by the protein transition or the need to create lower caloric foods, for instance. In this context, the use of pulse flours is a new strategy to replace (part of) wheat flour in wheat-based foods such as pasta, bread, and other bakery products [37–39] aiming to increase protein content for example. Additionally, consumption of foods made from ingredients of different plant sources results in an enriched nutrient intake [39]. Both purified (e.g. protein isolates, isolated cell fraction) and whole ingredients (e.g. whole pulse flours) of pulses are currently under investigation, yet the isolation and/or purification of pulse-based ingredients is accompanied by the production of several waste streams questioning the sustainability aspect of these food ingredients [40]. Additionally, pulse flours can be produced from either dry, uncooked pulses or from cooked pulses [41]. When mechanically disintegrating dry pulse seeds, cell breakage will be the prevalent mode of action, while sufficiently cooked pulse seeds will present predominantly cell separation [9]. These two types of pulse-flours have a completely different microstructure which is directly linked with macronutrient digestion kinetics. While predominantly raw-milled flowers are used by industry at this moment, there is increasing research showing the advantages of using cellular flours (i.e. flours made of cooked pulse seeds) in food products since the natural encapsulation of nutrients is preserved in that case [41], creating opportunities to tune digestion phenomena [42–44] and *in vivo* responses [39,45].

In this context, it is very important to realize that nutrients can behave differently in their isolated form compared to when they are present in a food or meal [1]. Guevara-Zambrano et al. [46] studied the impact of different protein microstructural organizations in the presence of emulsified oil droplets on both lipid and protein digestion kinetics. A direct link was observed between the level of accessibility of protein for digestive enzymes and protein digestion kinetics. In other words, naturally encapsulated protein can be used as tool to delay its digestibility. Additionally, the presence of the protein matrix delayed lipid hydrolysis, showing the importance of not only studying nutrients in their isolated form. A similar observation was made by Calvo-Lerma et al. [47], yet the impact of the protein content seemed negligible for foods with a high lipid content. More and more research focusses on investigating and understanding these nutrient, food, or even meal interactions [30] which remains a highly relevant research topic.

4 *In vitro* approaches to investigate digestion

Throughout the years, diverse approaches have been used to simulate digestion *in vitro* [48]. Static *in vitro* models are predominantly employed in that case, since they are based on *in vivo* observations, nonetheless being more standardized, less expensive, of higher throughput, and having less ethical constraints than *in vivo* models [48,49]. Static models only apply a single set of initial conditions and are thus unable to include any time dependency of the dynamic digestion process within a particular digestive phase [49]. Hence, semi-dynamic *in vitro* models are of interest as these models allow to make strategic choices on which relevant dynamic factors to include [50,51]. The dynamic nature of secretions (e.g. digestive enzymes, pH) and gastric emptying are two important aspects that impact digestion kinetics [50,52,53] as they modify microstructural properties, enzymatic activity, substrate-enzyme contact time, etc. Additionally, particular digestive compounds may interact with nutrients impacting their or other nutrients' digestibility/bioaccessibility. Interesting examples are the interaction of pectin with lipase and/or bile salts reducing the availability of these digestive compounds for lipid digestion when used as emulsion stabilizer [54]. More recently, it was found that certain bean compounds also retain bile salts which might be linked to a cholesterol-lowering effect [55]. Overall, it is important to choose an appropriate *in vitro* digestion protocol for the research question in mind [56]. Moreover, the impact of food structure on oral processing cannot be forgotten [57,58]. Additionally, more and more *in vitro* fermentation protocols are available as well which can deliver additional insight into the nutritional impact of food structure as affected by food processing or food formulation [59,60].

Until now, most digestion studies focused on digestion conditions typical for healthy adults. However, there is a growing need to unravel the digestive fate of nutrients for specific populations with altered digestion conditions [61]. Examples are children, elderly, women, people with anorexia, obesity or that underwent bariatric surgery, whom have altered transit times, pH conditions, enzyme concentrations and/or bile salt concentrations [62–65]. These altered digestive conditions will impact nutrient digestibility and bioaccessibility and we need to engineer foods for these populations as well. Providing food according to the health status of particular populations appears an emerging research field to which food scientists could and should contribute [66,67].

5 Conclusions and future perspectives

Engineering approaches like food processing or food (re)formulation remain interesting tools to modulate the digestive fate of nutrients. In recent years, food scientists more and more focused on the link between food processing, food (micro)structural properties and the final nutritional functionality of a food product or ingredient. Generally, when food (micro)structure gets lost by, for example, processing or formulating products from individual ingredients, the nutritional quality is largely altered. However, many effort is being made by food scientists to investigate the potential of intelligently designed new or reformulated foods to steer their digestive functionality. Since focus shifted from studying food composition towards studying food (micro)structure and its relation with nutrient digestibility and/or bioaccessibility, new tools should be developed to better quantify this relation. Additionally, there is a need to evolve from investigating single nutrients towards co-ingested nutrients in food and meal approaches spanning diverse subgroups of the whole population.

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CRediT roles

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