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REVIEW

Implications of static *in vitro* digestion of starch in the presence of dietary fiber

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Abstract Interest in understanding the digestion behavior of starch in the presence of dietary fibers is growing due to the ability of dietary component to control the release and absorption of glucose. This presents an outstanding opportunity to improve the quality of food products by incorporating dietary fiber into starchy food products. The physicochemical properties of different fibers and their behavior in the gastrointestinal tract (GIT) differ. To test the efficacy of these different fibers on starch digestion, static *in vitro* digestion models under conditions that mimic the human GIT are frequently used. Indeed, many efforts have been committed to the development of various static *in vitro* protocols for starch digestion. Though not considered as the gold standard in digestibility studies in food science and technology, static simulated models provide a useful alternative to *in vivo* techniques for rapid screening of the digestibility of food products under conditions that simulate the human GIT. This review presents the current status and development of digestion techniques for simulating digestion conditions in the human GIT, with particular interest on starch digestion in the presence of dietary fiber in the three phases of digestions including the oral, gastric and the intestinal steps. This summary can benefit investigators in developing static *in vitro* digestion models designed to simulate starch digestion with relevant values of the quantifiable parameters, including pH, enzymes and simulated digestive fluids.

Keywords dietary fiber, *in vitro* digestion, nutrition, simulation, starch

1 Introduction

It is well established that increasing the dietary fiber content of a food system is an effective way to deliver a low calorie diet and functional food products to consumers. Adequate dietary fiber intake offers several health benefits, such as a positive influence on the gastrointestinal physiology and lipid metabolism, bodyweight regulation and glycaemia control[1–5]. There is a considerable amount of research indicating the ability of indigestible polysaccharides to beneficially influence the physiology of the gastrointestinal tract (GIT), which is determined by the physiochemical properties and molecular behavior of the polysaccharides within the lumen of the GIT[3,6–8]. The interest in understanding the physicochemical properties of dietary fibers and their influence on the digestive processes of food within the human GIT has therefore taken center stage in the food research community. Food scientists are capitalizing on this knowledge to design food products that either inhibit or slow down macronutrient nutrient release from the food matrix and their subsequent absorption in the GIT[6,9–11]. By understanding the physicochemical properties of dietary fibers, this could be exploited to design food to deliver specific nutritional functions, such as enhanced postprandial satiety, the rate and extent of macronutrients (e.g., glucose and fatty acids) release in specific portions of the GIT and glycaemia control[3,12,13]. Some research recently demonstrated that the enrichment of biscuits and fruit juice with oat β-glucan improved postprandial satiety and this was related to the feeling of fullness induced by increased viscosity, which slowed down the rate of gastric emptying[6].

Analytical tools are required to test the efficacy of diverse dietary fibers in fiber-fortified food products that have been formulated to control starch digestion and glucose release for specific nutritional functions. Ideally, the gold standard to examine a newly developed fiber-fortified food should be *in vivo* test models using animals

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or human. Unfortunately, this testing model is usually unrealistic due to ethical restrictions and economic and practical considerations[14]. Apart from the high cost associated with human subjects, it requires considerable time and also is susceptible to considerable subject to subject variation. To overcome the constraints of *in vivo* models, static *in vitro* digestion methods are widely used to investigate the gastrointestinal behavior of newly developed functional foods including dietary fiber[7,14–18]. Typically, the techniques attempt to simulate physiological states encompassing the buccal, gastric and intestinal, and sometimes colonic, fermentation by considering factors including digestive enzyme types and their concentrations, pH, digestion time and salt concentrations[14].

The digestion of starch in the GIT involves three main phases: buccal, gastric and intestinal. Residual starch that escapes these phases undergoes fermentation in the colon[19,20]. In humans, maximum starch hydrolysis is accomplished in the duodenum, and by the salivary enzymes in adults and infants respectively[21,22]. In human nutritional studies of a variety of dietary fibers, *in vitro* digestion methods serve as rapid and less expensive tools for screening newly developed functional food products to identify potential candidate for more rigorous animal or human studies[14,23]. By operation, *in vitro* digestion models are categorized as static and dynamic. Though the static models are incapable of adequately mimicking the dynamic physiological processes food undergoes in the human GIT, particularly the mechanical actions of the mouth and the stomach, they are widely used due to convenience and ease of operation[24].

Several investigators have used static models to investigate digestibility of starch with or without dietary fiber[6,7,11,25–28]. These static models were either modified or unmodified versions of the widely-used protocol developed by Englyst et al.[29] to classify starch according to its rate of digestion with a controlled pancreatin and amyloglucosidase hydrolysis. This review aims to provide an overview of simulated static digestion models used to study the influence of various dietary fibers on the digestibility of foods high in starch. This is achieved by considering recent major advances of research in the context of *in vitro* static digestion. Starch hydrolysis in the presence of dietary fibers and the nutritional implications are also reviewed.

2 Brief overview of *in vitro* digestion models

Fundamentally, any *in vitro* digestion model aims to mimic all the digestive processes that occur in the different sites of the GIT, taking into account the digestive fluids, digestion time, and concentrations of enzymes and salts. Dynamic *in vitro* digestion models, such as *in vitro* dynamic gastrointestinal digestion system (DIDGI developed by National Institute for Agricultural Research (France)[30], Institute of Food Research (UK) and Netherlands Organisation for Applied Scientific Research[31], and simulator of gastrointestinal tract (SIMGI) developed by the Spanish National Research Council[32], are able to simulate dynamic components of the digestion process, including transport of digested food, adjustable enzyme concentrations and pH changes over time. However, a survey of the literature indicates that mostly static models have been used. Further evaluation of these studies showed variation depending on the nature of the food matrix and parameters considered in the study. It was observed that most of the food samples tested in the presence of dietary fiber were starch or starch in a modified form. Moreover, foods including bread, biscuit, juices and meat that have been fortified with dietary fiber were also commonly tested in the studies reviewed. The *in vitro* digestion models used to study the digestion of starch, and related products, in the presence of dietary fiber differed operationally as detailed below.

(1) The number of phases considered in the digestion steps (i.e., buccal, gastric and intestinal phases). The best simulation of the digestion process requires that these phases are simulated with all phases considered in the process. However, researchers may or may not entirely simulate all the phases depending the objective of the study as well as the food matrix tested[14].

(2) The composition, pH and amount of the digestive fluids used in each phase of digestion: There is significant variation in pH in the studies reported[12].

(3) The activities or mechanical processes employed to mimic each phase in the digestion sequence and duration of the digestion process. This is very important when it comes to solid food digestions under simulated conditions[14].

Additionally, the food form or matrix (e.g., solid, semisolid and liquid) before the digestion process as well as the parameters measured in the experimental process (e.g., changes in viscosity and physical integration of the enzyme with fiber component) varied greatly[25]. Some static *in vitro* studies attempted to mimic digestion by employing all enzymes, and other biological molecules, usually involved *in vivo* digestion[18,20]. Others used enzymes such as α-amylase, and amyloglucosidase that are associated with starch hydrolysis[6,7]. For all static *in vitro* digestion studies, incubation at 37°C in a thermostatically controlled water bath has been used for all steps of the digestion sequence.

3 Simulated digestive fluids and associated enzymes

In vitro digestion is performed in buffer systems which are usually constituted to simulate the pH, chemical and biochemical compositions of the human digestive fluids secreted in the mouth, stomach and intestines by the associated glands. This section highlights the key

parameters that are necessary to consider when preparing simulated digestive fluids to simulate starch digestion in the human GIT.

pH of the secreted digestive fluids that mix and surround the food matrix varies greatly as the food moves through various phases of digestion; mouth about pH 7.0, stomach about pH 1.0–3.0, small intestines about pH 7.0 and large intestine about pH 5.5–7.0. These pH changes may lead to a considerable change in the rate and degree of starch digestion. In the lumen of the GIT, mostly the polysaccharides including dietary fibers and starch physiochemical properties (e.g., viscosity, solubility and surface activity) are determined by the pH of the digestive fluids[26,33]. Simulated digestive fluids are usually formulated to reflect this pH change at the various phases of the digestion sequence (Tables 1–3). The role of pH in static digestion models has been discussed in detailed in two previous reviews[12,51].

Apart from pH, the biochemical and chemical composition of digestive fluids are also vital parameters to consider in the design of any simulated digestion protocol. During static *in vitro* digestion of a food matrix in the presence of dietary fiber, the proposed models had wide variation in the constituents, such as enzymes and salts, and their

Table 1 Summary of oral phase conditions used for static *in vitro* studies

Food	Particle size reduction	Composition of simulated salivary fluid	Means of enzyme impregnation	Duration in oral phase	Reference
White bread, whole wheat grains, pasta, chick peas and potato	Chewing and manual chopping	Human saliva, phosphate buffer, pH 6.8	Mastication, mixing	2–30 min	[34]
Cooked rice grain	Homogenization by homogenizer	5 mg α-amylase, calcium chlorite, pH 6.5	Stirring with magnetic stirrer	15 min	[35]
Bread	Cutting and grinding with knife meat grinder	Mucin α-amylase, NaCl, KCl, $NaHCO_3$, pH 6.8	Pounding with pestle and mortar	30 s	[36]
Starch based filled hydrogels	Cutting and grinding with pestle and mortar	NaCl, NH_4NO_3 KH_2PO_4, KCl, $K_5C_3H_5O_7 \cdot H_2O$, H_2NCONH_2, $C_5H_3N_4O_3Na_2$, mucin, pH 6.5	Stirring	10 min	[37]
Emulsion stabilized with dietary fiber	Homogenization	NaCl, NH_4NO_3, KH_2PO_4, KCl, $K_5C_3H_5O_7 \cdot H_2O$, H_2NCONH_2, $C_5H_3N_4O_3Na_2$, mucin, pH 7.0	Magnetic stirring	10 min	[38,39]
Emulsion	Homogenization	1g α-amylase, urea, uric acid, mucin, KCl, KSCN, NaH_2PO_4, Na_2SO_4, NaCl, $NaHCO_3$, pH 8.0	Magnetic stirring	5 min	[40]
Concentrated methylcellulose oil/water emulsion	Stirring	Phosphate buffer, mucin, pH 6.5	Magnetic stirring	5 s	[33]
Deoil cumin dietary fiber mixed with potato starch		Phosphate buffer, pH 6.5	Shaking by orbital shaker	60 min	[41]
Cooked potato	Mixing using glass spatula	Fresh human saliva	Shaking	5 s	[19,42]

Table 2 Summary of simulated gastric conditions used for static *in vitro* studies

Food	Composition of simulated gastric fluid (SGF)	Container and stirring speed	pH and SGF volume	Duration of transit time/incubation time	Reference
Modified and native wheat starch	Pepsin, US pharmacopeia SGF	50 mL beaker, 60 $r \cdot min^{-1}$	pH 1.2, 20 mL	40 min	[19]
Bran protein and dietary fiber complex with starch	Na_2PO_4 buffer, NaCl, pepsin	100 mL beaker, 250 $r \cdot min^{-1}$	pH > 2.5, 52 mL	120 min	[17]
Dietary fiber rich pasta	Distil H_2O, pepsin	60 mL plastic biopsy pot, 130 $r \cdot min^{-1}$	pH 2.0, 31 mL	30 min	[18]
Guar gum mixed with starch	Pepsin, US pharmacopeia SGF	1 L jacketed glass reactor, 650 $r \cdot min^{-1}$	pH 1.2, 30 mL	30 min	[43]
Starch hydrogel corn starch	NaCl, pepsin, distilled H_2O	50 mL beaker, 100 $r \cdot min^{-1}$	pH 2.5, 25 mL	120 min	[39]
Cooked rice starch	Pepsin, US pharmacopeia SGF	500 mL, jacketed reactor, 350 $r \cdot min^{-1}$	pH 1.2, 200 mL	30 min	[35]
Rice starch with guar gum	Distil H_2O, pepsin	50 mL tubes	pH 2.0, 25 mL	30 min	[44]
Modified wheat starch gels	Pepsin, US pharmacopeia SGF	50 mL beaker, 60 $r \cdot min^{-1}$	pH 1.2, 20 mL	40 min	[45]
Cooked potato starch	Pepsin, US pharmacopeia SGF	500 mL vessel, 350 $r \cdot min^{-1}$	pH 1.2, 150 mL	30 min	[46]

Table 3 Summary of static *in vitro* digestion models used to study the intestinal digestion of starch in the presence of dietary fibers

Food	Composition of simulated intestinal fluid (SIF)	pH and stirring speed	Parameters investigated	Digestion duration	Nutritional significance	Reference
Rice grain cooked starch	Pancreatin, invertase, amyloglucosidase sodium acetate buffer	pH 6.8, 350 r·min^{-1}	Effects of α- amylase and oral digestion on glucose release Effect of grain type and particle size glucose hydrolysis	270 min	Provide understanding of the influence of chewing and particles on glucose release rate. This could allow manipulation of the starchy food to change the digestion rate To quickly screen the digestibility of different grains in order to make appropriate nutritional recommendations	[35]
Fiber rich pasta	Pancreatin, amyloglucosidase Sodium maleate buffer	pH 7.0, 130 r·min^{-1}	Fiber influence on starch digestion Synergistic influence of difference fibers on starch digestions	120 min	For quickly screen functional dietary fibers for nutritional purposes To provide knowledge on how to mix fibers for good nutrition outcomes	[18]
Tapioca starch mixed with different dietary fibers	NaCl, $CaCl_2$ pancreatin	pH 6.5, rheometer shear rate 60 per second	Influence of viscosity on digestion rate Modification influence on digestibility Physicochemical properties of dietary fibers Dietary fiber influence on starch digestibility	180 min	For the modification of high glucose yielding food to minimize digestion rate To quickly screen newly formulated functional foods for nutritional purposes Provide information for nutritional claims Understand the mechanisms and nutritional role of fibers in the digestion processes	[26,43]
Starch mixed with different concentrations of different fibers	Sodium acetate buffer, pancreatin, amyloglucosidase	pH 6.0, 750 r·min^{-1}	Influence of fiber concentrations on starch digestibility Effect of dietary fiber source on digestibility of starch. Influence of starch on digestion in the presence of dietary fiber	240 min	Provide information to estimate the right amount of dietary fiber for nutritional benefit To quickly screen functional dietary fiber for nutritional counseling To identify sources of dietary fiber with nutritional benefits	[8,47]
Starch hydrogel	Bile extract, calcium chloride, phosphate buffer, pancreatin	pH 7.0, 100 r·min^{-1}	Influence of starch on lipid digestion	120 min	Emulsification of fat for functional food products For the development of controlled release of bioactive oil, such as PUFA	[39]
Modified wheat starch	Pancreatin invertase amyloglucosidase	pH 6.8, 60 r·min^{-1}	Influence of starch modification on glucose release rate Rheological properties on digestion rate	120 min	Modification of starch to slow down digestibility and glucose release for functional foods To quickly screen newly developed modified high starch foods	[19]

(*Continued*)

Food	Composition of simulated intestinal fluid (SIF)	pH and stirring speed	Parameters investigated	Digestion duration	Nutritional significance	Reference
Fiber rich cake, wheat gel with apple fibers	Pancreatin, amyloglucosidase, bile, sodium phosphate buffer	pH 6.5	Identification of cake quality based on glycaemic response Influence of fiber on nutritional quality of cake Effect of fiber in cake on glucose release rate	120 min	To quickly screen newly developed product for nutritional functions Control of glucose release and absorption Improvement of GIT physiology and health Predictive glycaemic index values for nutritional counseling	[15,48,49]
Fiber rich Biscuit	Sodium acetate buffer, amyloglucosidase, pancreatic amylase	pH 5.2	Influence of fiber on biscuit digestion Determination of predictive glycaemic response	180 min	Production of nutritional products with low glycaemic index Control of blood glucose through slow release of glucose for starch Improvement of gastric motility Improve postprandial glycaemic response	[50]
Durum wheat with Starch	Lecithin, cholesterol, sodiumtaurochlate, sodium glycodeoxycholate, sodium chloride, calcium chloride, potassium chloride, trypsin, pancreatic amylase, colipase, pancreatic lipase, chymotrypsin	pH 7.0, 170 $r \cdot min^{-1}$	Effect on starch digestibility Effect of fiber particle size on starch digestion Influence on bioavailability of glucose in the presence of durum wheat fiber		For development of functional products for blood glucose control To provide bioactivity in the body Control of glucose release and absorption	[10]

concentrations, as well as other biomolecules that constitute the simulated digestive fluid. Moreover, the reported protocols used to mimic the mechanical action applied to reduce the particles of the food (particularly solid foods) and the duration of digestion are different[23]. This suggests that *in vitro* digestion cannot exactly simulate *in vivo* digestion. For *in vitro* digestion, the widely used enzymes including α-amylase, amyloglucosidase, chymotrypsin, lipase, pancreatin and pepsin, and occasionally colipase. These enzymes usually come from variety of sources, including humans, animals and plants[23]. However, commercial enzymes widely used in the *in vitro* static investigations are usually extracted from pigs, oxen and rats, and occasionally from human volunteers. These different sources undoubtedly have a significant influence on the activity and characteristics of the enzymes used[14,23]. Though enzymes from human sources are considered ideal in nutritional studies in the context of static *in vitro* digestion models, they are rarely used in most laboratories due to their extremely high cost relative to the other sources.

Further analysis of the literature reviewed in this article indicated that the choice of enzyme in the digestion models tends to depend on the major food component. Also, the parameters being considered in the study are key to the choice of enzymes. For instance, Dhital et al.[7] exclusively used α-amylase to investigate the mechanism of the inhibitory effect of cellulose on digestion of corn starch, and Repin et al.[26] used pepsin and pancreatin (containing α-amylase, lipase and protease) to evaluate the impact of viscosity of four different types of soluble fiber on tapioca starch hydrolysis. In their study of the influence of different dietary fibers on digestion of cooked starch, Bai et al.[47], used pancreatin and amyloglucosidase.

In view of this wide variation in static *in vitro* digestion models, it can be difficult to compare results. To minimize the impact of these differences on static *in vitro* digestion methods, COST Infogest (Infogest website) brought together over 340 scientists (food, nutrition and physiology researchers) from 130 research institutes in over 37 countries, to develop an international consensus, standardizing the various static *in vitro* digestion protocols in common use among researchers. Recommended enzymes types, activities and their concentrations, and duration of hydrolysis in the digestion sequence have been published[14].

4 Static *in vitro* digestion of starch in the presence of dietary fiber

As dietary fiber passes through the GIT, it influences the

process and physiology of digestion. It is noteworthy that this influence is, however, dependent on the type of dietary fiber and its origin. The section considers the digestion of starch in the presence of dietary fiber in the three major digestion phases.

4.1 Buccal phase *in vitro* digestion

This is the shortest phase of the digestion sequence. *In vivo*, food is subjected to a series of mechanical and chemical changes in the oral phase before swallowing. By chewing, solid or semisolid food is broken down into smaller pieces and mixed with saliva. Thus, hydration and lubrication of food occur in the oral phase, where the food (either solid or liquid) is mixed with saliva. This paves the way for the salivary active biomolecules to interact with the food matrix and cause structures to either form or breakdown[52,53]. In addition to particle size reduction and lubrication of the food, the enzymes in saliva may be activated to initiate hydrolysis of starchy foods. According to *in vivo* studies, optimum hydrolysis of digestible carbohydrates in infants is accomplished in the mouth[12], while in adults this occurs in the duodenum. It has been demonstrated by static *in vitro* models that 25% and 50% of starch in pasta and bread, respectively, can be hydrolyzed in the oral phase of the digestion sequence[34,54].

Few investigators have designed simulators to mimic the mastication of food in the mouth[53,55,56] and solid food is often homogenized with a blender to form a paste-like matrix before simulating the digestion process in the mouth[35,57]. Magnetic stirrers, shakers and jacketed reactors have been used to simulate the mixing actions of the mouth during mastication. Stirring or shaking speeds used in published studies have varied widely (Table 1). Depending on the type of food matrix under investigation, the mechanical action used may influence the outcome of the digestion. The important role of α-amylase in the hydrolysis kinetics of bread has been reported for *in vitro* models[36]. In their study of the influence of in mouth processing on lipid hydrolysis and beta carotene bioavailability in starch hydrogel, Mun and McClement[37] observed that the mechanical process could influence the gastrointestinal outcome.

Simulated salivary fluids (SSF) of about pH 7.0 used in the various studies have varied widely depending on the focus of the study and the food matrix used (Table 1). While some investigators have used only simple buffers without extra components, others constituted SSF that included all the components usually found in human saliva, including acids, buffers, enzymes, minerals and mucins (Table 1). The latter is usually the case when it comes to food technology research, where scientists attempt to closely simulate human saliva. Human saliva produced by healthy individuals has also been used for static *in vitro* digestion models, where saliva from the healthy volunteers is collected after through oral washings. For instance, Woolnough et al.[34] demonstrated in a static *in vitro* digestion model that exposure of different starchy food to human saliva produced glucose release curves that were significantly different from those obtained with simulated salivary fluids. However, during the *in vitro* static digestion intestinal phase, no significant difference was observed because pancreatic amylase activity overwhelmed that of the salivary amylase.

In static *in vitro* digestion, designed to investigate carbohydrate glycaemic index, transport and absorption of glucose and other outcomes, the oral phase is rarely considered, even though it is well known that the digestive processes begins in the mouth, where the release of some nutrients, particularly glucose, from the food matrix begins[53]. Based on the literature reviewed, an overwhelming number of investigations skipped the oral phase of the digestion sequence probably due to the inability to adequately control the short duration of the process in the oral phase. Also, this might be due to the fact that enzymes in the subsequent phases, particularly in the intestinal phase, are capable of hydrolyzing the food component. The proposed standardized protocol[14] includes recommendations for appropriate dilutions, enzyme constitution and pH controls in the oral phase.

4.2 Gastric phase

In the gastric phase, the stomach basically functions as storage compartment and to deliver digesta to the small intestine in a controlled manner. *In vivo*, physical and physiological processes including peristalsis and pH changes occur in the stomach to further break down the larger solid food received from the mouth into smaller pieces in order to increase the surface area for optimum exposure to the digestive enzymes in the small intestine[58]. In the case of static *in vitro* digestion models, these important physiological dynamic processes that occur *in vivo* are not simulated. In general, nearly all the static *in vitro* models simulated the gastric phase of *in vitro* digestions by mixing food samples with a fixed volume of simulated gastric fluids (SGF) maintained at a simulated gastric conditions for a period of 90 to 120 min[14]. Sampling is either done at a specific time interval (e.g., every 30 min) until 120 min or at the end of the entire gastric digestion process before moving the gastric digesta to the simulated intestinal conditions for further digestion. The mixing which allows the complete exposure of the enzymes to the food matrix can be performed at fixed speed using a magnetic stirrer, shaker or jacketed reactors. The mixing speed used has varied widely in the studies conducted over the past few years (Table 2). Apart from pepsin and lipase, all other enzymes are inactive under the gastric environment due to its relatively low pH of about 2.0. The lipases frequently used in static *in vitro* models to simulate the human gastric lipase are biochemically

questionable. In their review of pH and gastric lipase activity *in vitro* digestion models, Sams et al.[51] reported that gastric lipase from humans was biochemically unique, differing from lipases from other sources, and could be stable and function in the pH range of 2.0 to 7.0 with optimum activity of 4.0 to 5.4. According to our examination of the literature, pH used in the gastric phase digestion ranged from 1.2 to 3.0 (Table 2). The α-amylase in the digesta received from the oral phase is inactivated below a pH of around 2.0 in the gastric phase.

Also, the static digestion models that have been employed to investigate structural changes and physiochemical properties of starch in the presence of dietary fiber have not considered peristaltic movement or movement of the digesta. Whether the static gastric digestion is sufficient depends on the influence of each physiological parameter on the digestion and envisioned end point. Often researchers ignore the gastric phase[15,18,48,50,59] in their static *in vitro* digestion models for starch digestion, assuming that the gastric phase is completely is overridden by small intestinal digestion. Also it is assumed that amylase is inactivated under gastric conditions, so samples with starch as the major nutrient to be hydrolyzed do not necessarily need gastric phase simulation. However, the systems that omit the gastric phase and the gastric enzymes (e.g., pepsin, trypsin and chymotrypsin) may not be sufficient to simulate complete gastrointestinal hydrolysis of starch. This is because the preliminary digestions of starch by gastric trypsin and chemotrypsin are reported to further trigger pancreatic amylase activity on cooked rice and starch gels *in vitro*[20]. Similarly, gastric lipase has been reported to be a key trigger of further pancreatic lipase activity on lecithin-stabilized emulsions *in vitro*[12,14]. Therefore, whether omission of the gastric phase influences the digestion process depends on the food matrix and the parameters being investigated. The recently-proposed harmonization of the various static digestion models is paramount for the meaningful comparison of results from various research groups. For the standardized *in vitro* starch digestion, it has been proposed trypsin, pepsin and chymotrypsin be used as the major protein hydrolytic enzymes[14].

Apart from mixing methods, static *in vitro* digestion studies also differed in the constituents of the simulated digestive fluids, digestion time, and the enzyme type and concentration. Table 2 summarizes gastric conditions that have been used for static *in vitro* digestion of starch. It is recommended that SGF should be constituted giving consideration to the relevant enzymes and salts that are present in human gastric fluids[14]. Similarly, the pH of the SGF should be adjusted with the appropriate electrolytes to a pH of around 2.0.

4.3 Intestinal phase

The intestinal phase is key for the final digestion of most of nutrients in the food matrix, since it is the phase where remaining starch is hydrolyzed into glucose for absorption. Unlike the oral and the gastric phases, which may be omitted in some cases, most of the static *in vitro* models reviewed were conducted under intestinal conditions. Primarily, the GIT operates in a dynamic fashion, with movement and digestion of food, and absorption of nutrients occurring concurrently in a complex environment with mixing and transport along the digestion sequence[58].

For *in vivo* models, chyme received from the gastric phase is physiologicalally neutralized. While the secreted bile salts and phospholipids function to emulsify lipid particles in the chyme, sodium bicarbonate neutralizes the highly acidic chyme to create a suitable environment for the activity of the secreted intestinal enzymes[12]. In static *in vitro* models, the transferred chyme received from the gastric phase is diluted with a known volume of simulated intestinal fluids (SIF) and subjected to mixing with a magnetic stirrer[15,50], shaking[26] or jacketed reactor[60] at a constant speed for a set period. Apart from not being able to simulate the exact physiological process *in vivo*, static *in vitro* digestion models for the intestinal phase *in vitro* digestions are unable to eliminate digestive products, which may impede enzymatic activity[14].

The simulated intestinal gastric fluids that have been used in static *in vitro* digestion models vary in their constituents with pH around neutral (Table 3). While basic SIF are often constituted to have a mixtures of enzymes (pancreatin) and bile salts with a neutral pH, more complex SIF are formulated to contain buffers, salts, protein and enzymes to more closely simulate *in vivo* intestinal fluids[12]. The composition of the SIF depends on the nature and type of food matrix to be digested. Accordingly, several researchers have composed SIF differently to suit the samples being investigated[18,46,48,61] (Table 3). *In vitro* models designed to investigate the digestion of starch in the presence of other dietary components have mostly only used intestinal phase simulation, but some have included this in combination with other phases of the digestion sequence[62–64]. Any starch that is not digested and dietary fibers move to the larger portion of the duodenum, where they undergo fermentation to release compounds that have various biological functions in the body.

5 Nutritional relevance of starch hydrolysis in the presence of dietary fibers

One important defining and unique feature of any dietary fiber, irrespective of its source, is that its resistance to the human digestive enzymes. Though indigestible, dietary fiber has long been recognized as an important ingredient in the human diet. Dietary fiber has been defined as polysaccharides, such as cellulose, gums, pectin, inulin and hemicellulose, with ten or more monomeric units that are incapable of being hydrolyzed by the human digestive

enzymes[65–67]. Physicochemical properties of dietary fiber, which define its functional properties in the GIT with consequential impact on food digestion are determined by the fiber origin and its preparation method. In the light of this, dietary fibers from various sources have been investigated to evaluate their influence on the digestibility of starch by static *in vitro* methods[15,50,66,68] before the use of rigorous *in vivo* models using animals and humans. Successful candidates could be used to develop functional food products for improving human nutrition.

Consumption of a diet with sufficient dietary fiber is recognized as a means of reducing the risk of cardiovascular diseases and diabetes[3,69]. The mechanisms by which these beneficial effects are associated with dietary consumption include increase in viscosity caused by the presence of the fiber (particularly soluble fibers)[4,26,69,70] in the GIT and the ability of the fiber to bind to endogenous enzymes and inhibit their activity[7]. The authors of these studies have associated an increase in viscosity in the presence of fibers in the diet with a reduced rate of gastric emptying, glucose release and absorption. Several dietary fibers including cellulose and its derivatives (e.g., alginate and β-glucan, gums, inulin and pectin)[1,6,15,26,70] have been investigated by static *in vitro* digestion to provide an understanding of their influence on the digestion of starch and absorption of released glucose. In this way knowledge of functional foods can be obtained to meet the specific needs of consumers.

From the nutritional perspective, static *in vitro* digestion models offer a quick and less expensive way for initial investigations and screening of new functional ingredients. It has been demonstrated in many *in vitro* studies that during the digestion of starch, the presence of the fiber may interfere with enzymes by physical entrapment and binding, which can reduce their availability for digestion[62]. However, depending on dilution factors, concentrations and inherent physicochemical properties of the dietary fiber involved, the influence on the digestion of starch can differ. For insoluble fibers such cellulose, it is known that the fiber component physically binds to the enzyme and reduces its availability to degrade starch[7]. In the case of soluble gel forming fibers, such as gums, viscosity is key to the glucose release rate and absorption[26,69]. The relevance of static digestion models in nutrition and food science continues to promote the development and growth static simulated digestion (Table 3).

6 Conclusions

Studies conducted over the years that have focused on static digestion of starch in the presence of dietary fiber or starchy food fortified with fiber were critically examined to identify key parameters to consider in designing static simulated digestion models. This review of the literature found that static *in vitro* digestion models have been extensively used to examine food products in many digestion simulation studies. Also, it was found that the majority of the studies used enzymes of plant and animal origin, rather than of human origin. For simulated digestion of starch in the presence of dietary fiber, the intestinal phase was the most simulated followed by oral phase simulation. Nutritionally, the development of new functional fiber-rich food products has heavily relied on these simulated static models for initial evaluation. However, the models are constrained by lack of standardization, making it difficult to compare results even between similar static simulated digestion models. Reducing the impact of such differences in the digestion models used by different researchers should be achieved by harmonization of the important parameters commonly used to simulate the digestion conditions. Though an international consensus on standardization of the various protocols of static simulated digestions has been proposed, its acceptance and adoption are so far limited.

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This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

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