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Chapter

Binary Interactions and Starch Bioavailability: Critical in Limiting Glycemic Response

Veda Krishnan, Monika Awana, Debarati Mondal, Piyush Verma, Archana Singh and Shelly Praveen

Abstract

Limiting starch bioavailability by modifying food matrix dynamics has evolved over the decade, which further envisions low glycemic starch prototypes to tackle chronic hyperglycemia. The dense matrix of whole grain foods like millets and cereals act as a suitable model to understand the dynamics of binary food matrix interactions between starch-lipid, starch-protein & starch-fiber. The state and types of matrix component (lipid/protein/fiber) which interact at various scales alters the starch micro configuration and limits the digestibility, but the mechanism is largely been ignored. Various in-vitro and in-vivo studies have deciphered the varied dimensions of physical interactions through depletion or augmentation studies to correlate towards a natural matrix and its low glycemic nature. The current chapter briefly encompasses the concept of food matrix types and binary interactions in mediating the glycemic amplitude of starch. We comprehensively elaborated and conceptually explained various approaches, which investigated the role of food matrices as complex real food systems or as fundamental approaches to defining the mechanisms. It's a fact that multiple food matrix interaction studies at a time are difficult but it's critical to understand the molecular interaction of matrix components to correlate in-vivo processes, which will assist in designing novel food prototypes in the future.

Keywords: starch, digestibility, food matrix, binary interactions, glycemic response

1. Introduction

Starch is one of the major constituents of reserve food material, which serves as fuel for the human body. The calorific value of starch is 17.5 kJ/g, which is not only responsible for most of the metabolic functions but also acts as a crucial regulatory adjunct to control energy balance. Starch existed as the major dietary nutrient since time immemorial but the dietary transition with enriched refined products as well as carbaholic staples led to the unprecedented increase in the pre-diabetic and diabetic population with characteristic chronic hyperglycemia. Hence glycaemic response (GR) eliciting potential of food known as the glycemic index (GI) or glycemic potential (GP) are major aspects to understand as well as to fine-tune. In a food matrix,

starch bioavailability is modulated by the microstructure (cell wall, membrane, cell layers, granular size, etc.) as well as its dense composition (macro and micronutrients) [1]. Based on the interacting components food matrix interactions are classified as binary (two-component), ternary (three-component), and quaternary (four-component) [2–6]. Types of binary interactions and their effect on starch bioavailability are depicted in **Figure 1**.

Among these, a binary component has gained great importance and has been extensively characterized by component depletion or addition studies under in-vitro conditions [7, 8]. The observed low GP of whole grain foods like millets, pigmented rice has been well correlated with such matrix interactions present endogenously, while high GI has also been reported to lower by exogenous addition of such matrix components [9–11]. The state and types of matrix component (lipid/protein/fiber) which interacts at various scales have also been known to alter the starch micro configuration (repeat, reconstruct the sentence limiting the digestibility, result in lowering the glycemic response [9, 12].

Binary interactions have been majorly characterized using nutrient-sensing fluorescent probe-based confocal laser scanning microscopy (CLSM), where the proximity as well as encapsulating effect of matrix components limiting the starch hydrolytic metabolic enzymes have been observed [13, 14]. Further, the effect of

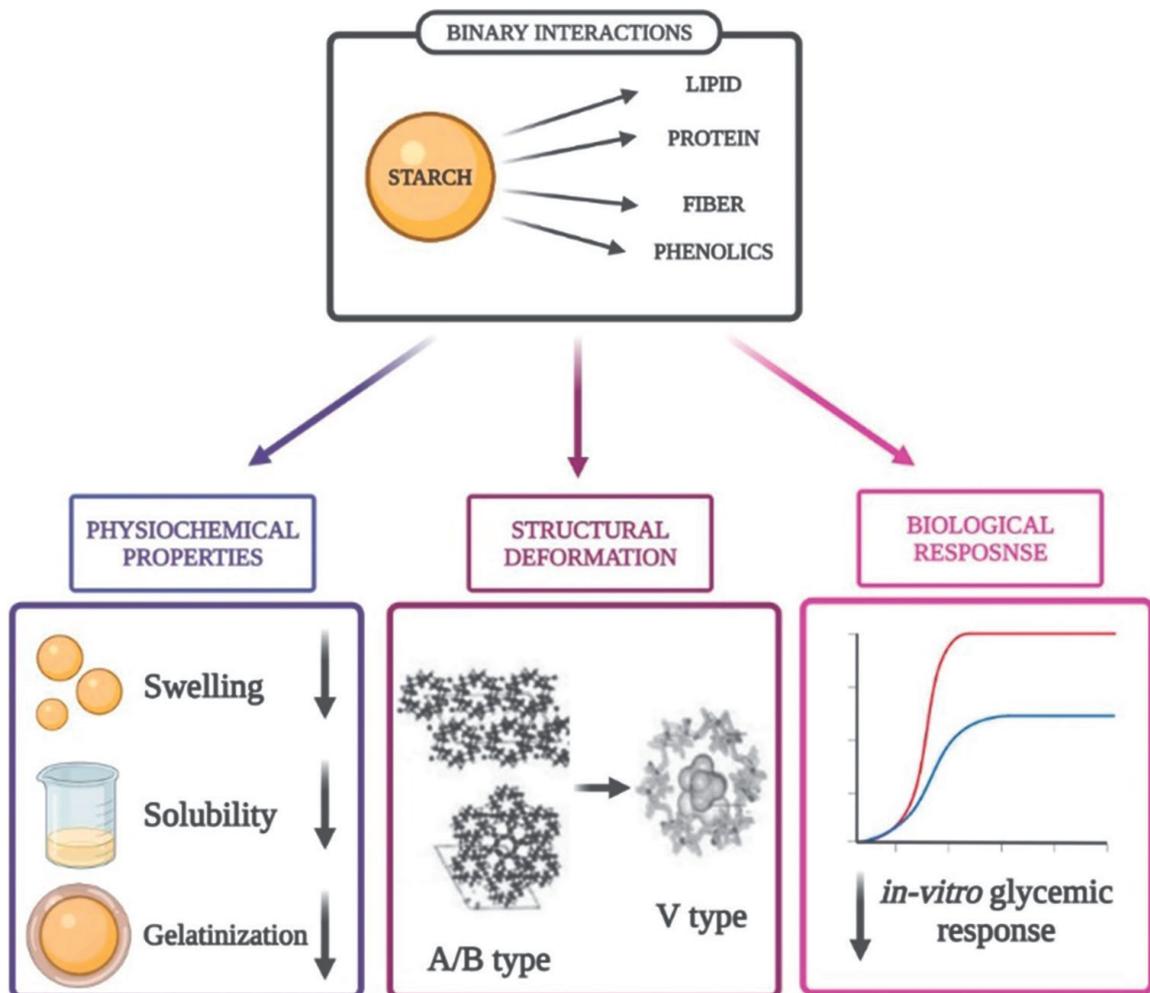


Figure 1. Types of binary interactions and its effect on starch bioavailability. Binary interactions modulate physiochemical, structural, and biological attributes limiting starch digestibility as well as ultimate glycemic response.

such interactions on starch functional aspects like hydration, enzymatic cleavage, or enthalpy have been delineated using differential scanning calorimetry (DSC). Scanning electron microscopy (SEM) assisted in revealing the structural alterations associated with starch in the matrix after component depletion or addition. Rapid visco analysis (RVA) revealed that viscosity and pasting parameters were found inversely associated with in-vitro starch digestibility. The effect of matrix components in retaining the matrix, granule stability, preventing the expansion of granules as well as limiting the glycolytic enzyme attack has been endorsed using this technique [15, 16]. Other than affecting the swelling of starch granules by reducing the contact with carbolytic enzymes, the effect of such binary interactions in altering the molecular configuration (digestion sensitive A or B type to resistant V-type) of starch was envisioned and characterized using X-ray diffraction (XRD) and Fourier transform infrared microscope (FTIR).

Among the binary interactions, the most relevant in limiting the glycemic amplitude includes starch-lipid, starch-protein & starch-fiber dynamics.

1.1 Starch-lipid interactions

Even though well compartmentalized, starch and lipids do interact endogenously in real food systems. Lipid content ranges from 0.2–7% in cereals, with the least reported in rice and maximum reported in the case of oats & pearl millet [17]. A balanced distribution of neutral, glycol, and phospholipids along with free fatty acids have been reported in most of the food matrices, assist in energy as well as membrane structure & functions [17]. Curiosity towards food matrix interactions underlined a striking correlation between high lipid content [18] and low GR, which initiated binary (starch-lipid) interaction studies [7, 8]. Endogenous and exogenous lipid content have found to have low in-vitro starch digestibility along with superior resistant starch (RS) fraction. The effect of endogenous and exogenous lipid types have recently shown to have an effect in increasing starch-lipid complexation enriching RS content in red rice [9, 10]. Ye et al. [11] suggested that among lipids and proteins, starch digestibility is most affected by lipids as it affects swelling of granules, reduces the contact with carbolytic enzymes as well as alters the molecular structure from A-type into resistant V-type pattern. The long hydrophobic tail of lipid entering the cavity-like structure of amylose enables starch to form a stable complex, thereby hindering the accessibility of starch to enzyme attack [11]. In the case of mung bean flour, in-vitro starch digestibility and GI were increased significantly when endogenous lipids were removed [19]. Previous studies by Panyoo et al. [12], Krishnan et al. [9] have mentioned that stable starch-lipid complex results in a twist in digestibility phenotype into a digestion resistant fraction (RS-V), which caters to the gut microflora. As stated above, Copeland et al. [20]; Wang and Copeland [21] suggested this inclusion complex of starch-lipid also has an immense role in the food industry such as lowering solubility, swelling power, starch gelatinization, retrogradation, and enzyme action.

Starch-lipid complexes can exist inherently within the food matrix, or they may be produced by exogenous applications. A study by Obiro et al. [22]; reported that this complexation is mainly influenced by non-covalent interactions (hydrogen bonds, hydrophobic interactions, van der Waals interactions, and so on). Hydroxyl groups α -(1,4) are situated on the outer surface whereas methylene and oxygen groups present in the inner region of the complex strengthen the formation of starch-lipid

complexes. Considering all positive impacts of starch-lipid complex, there are few factors (chain length of amylose, amylopectin, fatty acids, degree of unsaturation) that mostly govern the degree of complexation [10]. Various researchers stated that amylose acts as the primary constituent to interact with lipid molecules, while few reports supported the role of amylopectin chain length to form the complex [23, 24]. It has been reported from various studies that starch-lipid complexability has been increased with the longer chain length/degree of polymerization (DP) which highlights the formation of crystalline structure [25]. In addition to the effect exerted by the chain length of starch components, processing conditions like cooking also affect starch-lipid interaction. Kaur et al. [8] suggested amylose-lipid complexation enhanced with amylose chain length and increased with cooking time. Experiments highlighted the stability of starch-lipid inclusion complex formation mainly based on the types of fatty acids accommodated inside the helical cavity [26]. Different reports exist on the type of fatty acid for stable starch-lipid complexation. One school of thought suggests that the stability of the S-L complex could be enhanced by increasing the aliphatic chain length of fatty acids as well as melting temperature (from 8–10). On the other hand, another dimension highlights that smaller carbon chain length fatty acids might be more soluble into the aqueous solution and less stable also [27, 28]. Tufvesson et al. reported C14 as the most stable conformation than C16 or C18 while other explained C16 or C18 is better in the case of complexability [28]. Therefore, saturated fatty acid (SFA) with increased chain length can easily form a stable complex which further affects enzymatic accessibility due to resistance against carbolytic enzymes. Studies over decades highlighted that only SFA can be able to form a strong stable S-L complex with increasing chain length in a temperature-dependent manner whereas an inverse relationship has been found for unsaturated fatty acid (UFA) [26, 28]. A report from Zheng et al. [29] stated that chain length and degree of unsaturation have a role in the compact structure of starch-lipid formation. In addition to this, Kawai et al. [30] & Meng et al. [31] revealed starch-UFA complex showed resistance by formatting a stable complex to digestive enzyme action. The degree of complexability of FA in the case of maize starch ranged from 11.60–26.31% according to Sun et al. report [32]. Moreover, it has been explained from Sun et al. [32] RS is also enriched with the degree of unsaturation from 0 to 2%. In addition, thermal properties are also greatly affected by this S-L complex. Thermal complexes are mainly classified into two types of complexes as type I (90–115°C), type II (115–130°C) depending on the melting temperature. Studies from previous research have already highlighted that developed type II complex is more resistant to the digestive enzymes as compared to type I complex [33]. But Sun et al. [32] unraveled that maize starch-linoleic acid (MS-LOA) primarily formed as type I complex while maize starch-stearic acid (MS-SA) belonged to type I & type II complex. The reason behind this could be the large steric hindrance associated with LOA than SA which showed less accessibility of enzymes and inhibits ultimate glucose release. Cheng et al. [34] also used molecular dynamics to study amylose and linoleic acid structural analysis and conformational changes during complexation. On the continuation with Cheng et al., recently another research group of Schahl et al. [35] revealed the molecular structural complex using ¹³NMR spectroscopy where they have taken quantum DFT approach affected by amylose size fragment and specific intramolecular hydrogen bonds. Hence, all the V-type complexes produced due to the addition of lipids act as a stable resistant structure against all digestive enzymes which further lowers glycemic response.

1.2 Starch-protein interactions

Proteins, mostly in the form of amino acids, and enzymes, are the predominant component in the food matrix, other than starch and fat [36]. Apart from the nutritional quality, proteins act as the major microstructural framework in a food matrix and hence also act as a physical barrier towards starch hydrolysis [37]. An interesting correlation among the reduction in insulinemic and glycemic responses by increasing the protein content in starchy crops led to the possibility of starch-protein interplay. Among the protein types, albumin, glutenins, and globulins aid in the gluing of protein bodies into a matrix enveloping the starch granules, which act as a barrier for starch digestion [38]. The existence of a protein barrier encircling the starch granule was validated using the pronase enzyme which dissociates the protein matrix and results in a considerable increase in-vitro starch digestibility [39]. Annor et al. [40] reported that the hypoglycemic characteristic of Kodo millet was related to the protein encircling the starch granules. Ren et al. [41] also reported that there was a fast increment in in-vivo GI and in-vitro starch digestibility of foxtail millet flour due to the lack of starch-protein complex after deproteination. Various studies have reported that the presence of gluten has an impact on the pace of starch digestion, resulting in reduced glycemic response [42, 43]. Gluten develops a visco-elastic and thick network that entraps starch granules, as well as a compact and stable structure that prevents starch granules from expanding and leaching during cooking, resulting in reduced accessibility of enzyme and slow-release properties [44]. To study the impact of protein removal from wheat products (bread) on blood glucose, healthy individuals were given meals of white bread prepared either from normal or gluten-free flour. It was observed that there was a considerable increase in blood glucose after consuming bread prepared from gluten-free flour. This led to an increase in digestion rate in-vitro and declined the starch mal-absorption in vivo as studied via breath-H₂ measurements, but this impact was not restored when the gluten was later added back to the gluten-free flour. The possible mechanism behind this may be all-purpose wheat flour is made up of granules with a starch core enveloped by a protein network that inhibits the hydrolysis rate in the small intestine lumen [45]. Recently, Lu et al. [46] revealed that in the small intestine, amino acids generated from enzymatic hydrolysis of rice protein inhibited the porcine pancreatic α -amylase activity. The protein content of rice flour was shown to be negatively associated with rapidly digestible starch (RDS) and slowly digestible starch (SDS), while positively with RS [47], on the other hand, the total protein content of rice grain was found to be inversely correlated with in-vivo GI [48].

Other than endogenous factors, processing (thermic/mechanical) has been found to have an effect in altering the level of interaction between protein-starch molecules, influencing the overall digestibility [49]. Pasini et al. [50] found that in-vitro digestion of wheat protein has been considerably reduced at elevated cooking temperatures (>180°C) due to the development of high molecular weight protein aggregates which are stabilized by strong irreversible linkages, distinct from hydrophobic and/or disulfide bonds that could be prevalent at low temperatures (100°C). Furthermore, it has been found that “appropriate” kneading/mixing promotes the development of a protein matrix (gluten) via disulfide linkages. Moreover, if extreme kneading/mixing is performed, the matrix loses strength as the linkages break and glutenin particles are fragmented into smaller fragments, which helps digestive enzymes access the starch and thus increases the starch digestibility [51].

Protein-enriched food formulations have also been found to impact the overall GI and thus assist in developing diabetic-friendly foods. Formulations based on proso millet starch and different protein mixtures (15% zein + 10% whey protein isolate + 15% soy protein isolate) reported that protein types reduced the RDS levels and enhanced RS levels from 4.49% to 11.73%. The blend comprising of corn starch (10%) and whey protein isolate had a considerably higher concentration of RS and low RDS as compared to pure corn starch. This could be due to the increased protein matrix enveloping starch networks, preventing amylolytic attack. When soy protein was added to maize starch, RDS was reduced while SDS and RS were increased [52]. The addition of 51% rice protein in wheat starch along with cellulose reduced RDS level, whereas the addition of protein from pea proteins (82%), maize (95%), soy (94%), and wheat (86%) did not affect RDS levels [13]. Bio-mimicking interactions in corn grains using microencapsulation of corn starch by zein protein have been reported with lowered starch digestibility [53]. Furthermore, starch coupled with amino acids or protein via the Maillard process has been demonstrated to limit the starch swelling, solubility as well as digestion rate [54], however, potential negative effects due to glycated product consumption must be examined in detail [55].

1.3 Starch-fiber interactions

Dietary fiber (DF), which consists primarily of non-starch polysaccharides found in plant cell walls, is an essential part of the food matrix [56]. DF types present in any food matrix are classified based on their water solubility and fermentability. Lignin, cellulose, and hemicelluloses are the major water-insoluble DF that get less fermented while the water-soluble DF includes pectin, mucilage, and gums and gets fermented properly in the small intestine [57]. Among the types, insoluble DF has been reported to be more useful in decreasing the GI as compared to their soluble fraction [58] as most of the common cereals contained a low level of naturally occurring soluble DF [59]. The endogenous fibers encircle the starch granules forming a starch-fiber network in the matrix, bio-mimicking an intact microstructure (plant cell/tissue) result in reduced enzyme accessibility and altered digestibility. Dense matrix composition in fiber content has been positively correlated to minimal postprandial GR after consumption in the case of barley, wheat, psyllium husk, and oats. This has been majorly attributed to the effect of insoluble DF in reducing starch bio accessibility as well as bioavailability [60, 61]. On the other hand, soluble DF like inulin has been found to form a protective barrier surrounding the starch granules, reducing starch swelling and release of amylose thus resulting in low viscosity values. This reduced the accessibility of starch-degrading enzymes that affect the *in vivo* starch digestibility and GI [62]. Among the studied types, β -glucan and guar gum have been reported to reduce the enzyme diffusion kinetics and thus the rate of carbohydrate digestion, eventually resulting in to slow down the gastric emptying and lower the liberation and absorption of glucose in the small intestine [63, 64]. The endogenous presence of β -glucans (native-form) in oats have been found to have an enveloping role towards starch and protein, thus reducing the enzyme accessibility, in turn, lowered starch digestibility and postprandial glycemia [65].

Endogenous presence, as well as exogenous addition of cellulose (insoluble fiber), has considerably reduced the α -amylase activity via mixed-type inhibition resulting in lowered *in-vitro* starch digestion [66]. The reduction of α -amylase activity was found to be positively linked with cellulose content, and α -amylase was found to be non-specifically linked on the surface of cellulose, reducing starch hydrolysis.

Interaction study between pectin and digestive enzyme (amyloglucosidase) showed a similar pattern, where pectin resulted in the conformational alteration in an enzyme that impeded substrate access and slower digestion rate of long amylopectin chains [67]. Luo and Zhang [68] aimed to mimic the microstructure of endosperm tissue by constructing a starch in a whole-grain-like structural form using calcium-induced alginate gelation in the presence of β -glucan and starch.

Processing strategies, as well as formulations with exogenous addition of fiber types, have also been found to reduce the in-vitro starch digestibility and GI of foods [69]. The addition of fibers like xanthan gum, glucomannan, and agar in rice lowered the starch digestibility in-vitro and in-vivo [70, 71]. However, no relationship was observed between native fiber content (0.5%) and in-vivo starch digestibility in rice, even though the fiber level was certainly too less to have any influence on starch digestion [48]. Reduction in blood glucose [72] and in-vitro starch digestibility [73] was observed in wheat products after adding β -glucan. β -Glucan has been assumed to improve viscosity, which could have lowered the rate of gastric emptying [72] and lowered the rate of diffusion of starch digestive enzymes. Vegetables like *Moringa oleifera* leaves and okra were found to reduce the glycemic response of various foods. When 10% okara was added to rice noodles, blood glucose levels significantly reduced [74]. Broccoli fiber addition in a potato diet has proved to assist in decreasing the GI by increasing RS content [75]. However, a 30% decrease in GI in the same sample was observed when studied in-vivo. Hardacre et al. [76] showed that fibers with comparable viscosities resulted in variation of in-vitro starch digestion and hypothesized that few fibers may inhibit certain enzymes in a non-competitive manner as a chemical barrier. An interesting observation reported by Sciarini et al. [69] was that, the addition of up to 5% soluble (Inulin) and insoluble fibers (oat fiber and type IV RS) enhanced the starch digestibility in GF bread, while further increase resulted in a reduction in starch digestibility. The initial observed increase could be due to the altered bread crumb structure while a higher percentage of fiber could have established a staple starch-fiber network and thus reducing the digestibility. The impact of RS addition in pasta structure on native starch digestibility was studied by Gelencser et al. [77]. They reported that kinetic characteristics were not considerably variable between the control and RS-added samples, whereas the starch digestibility was considerably low in the RS-added samples, signifying a decrease in absolute glucose release during amylolysis. The addition of RSII (7.5%) and RSIV (10%) in pasta showed a decrease in in-vitro starch digestibility and GI [78]. Furthermore, larger DF concentrations also might play role in confining the starch inside the pores, preventing its hydrolysis. Mkandawire et al. [79] discovered that adding up to 50% cellulose (w/w starch) to sorghum flours had no significant impact on the RS level. DF, on the other hand, enhanced the solution viscosity in-vivo, and therefore may slow starch degradation by restricting enzyme mobility, as gums do, and hence slowing digestion rate in total [63].

In this direction, several animal studies have been carried out to study the effect of adding fiber on starch bioavailability or glucose release. The supplement of insoluble cereal DF from oat leads to enhanced insulin sensitivity in obese mice [80]. Further studies conducted by Weickert et al. [81] revealed that oat DF and purified wheat can enhance the postprandial insulin secretion hormones which further improved the postprandial carbohydrate metabolism. The high level (500 mg/kg body weight) of oat β -glucan or 4% barley β -glucan resulted in considerable enhancement of insulin resistance in insulin-resistant mice model and the impact was concentration-dependent [82, 83]. β -Glucan was found to inhibit the intestinal disaccharides' activities

in-vitro and in-vivo, which led to slow starch digestion rate [84]. In the diabetic mice model, β -glucan considerably repaired and increased the integrity of pancreatic islet β -cell and tissue structures [85]. Overall, the type and concentration of fiber have a customized effect on the food matrix. Comprehensive list of various food components added to starch and their effect on starch digestibility is tabulated in **Table 1**.

S. no.	Food component added	Added to starch	Impact on starch digestibility	Reference
Lipid				
1.	Cooking fats (ghee, coconut oil, virgin coconut oil, rice bran oil) (2.5%)	Rice starch (white, black, red)	↓	[9]
2.	Linoleic acid (0.75%)	Arrowhead tubers starch	↓	[86]
3.	Ascorbyl palmitate (10%)	High amylose maize starch, potato starch	↓	[87]
4.	Trans-oleic acid, cis-oleic acid, cis linoleic acid (1%, 3%, 5%)	Rice starch	↓	[87]
5.	Palmitic acid (0.5%)	Waxy rice starch	↓	[88]
6.	Oleic acid (1%, 2%, 3%) & linoleic acid (2%, 4%, 6%)	Rice starch	↓	[89]
7.	Linoleic acid, monomyristyl glycerol (0%, 0.5%, 1%, 1.5%, 2%, 3%, 5%)	Maize starch	↓	[90]
8.	Oleic acid (4%)	Native rice starch	↓	[91]
9.	Palm oil (5%, 10%)	Arrowroot starches	↓	[92]
10.	Lauric acid (1.5%)	Wheat starch	↓	[93]
11.	Oleic acid (0.05%)	Native potato starch	↓	[94]
12.	Dodecanoic acid, tetradecanoic acid, octadecanoic acid (1%, 3%, 5%)	Native rice starch	↓	[95]
13.	Decanoic acid, palmitic acid (10%)	Native maize starch	↓	[32]
14.	Lauric acid, stearic acid and glycerides (glycerol monolaurate, glycerol monostearate) (0.18%)	Wheat starch	↓	[93]
15.	Palmitic acid (0.3%)	Maize starch	↓	[1]
Protein				
16.	Rice globulin (2%)	Rice starch	↓	[15]
17.	Common bean (15%, 30%, 45%)	Wheat semolina	↓	[96]
18.	Gluten (2%)	Wheat flour	↓	[97]
19.	Beans (10%, 20%, 30%)	Semolina flour	↓	[98]
20.	White beans (15%, 30%, 45%)	Rice flour	↓	[99]
21.	Rice protein (51%)	Wheat starch	↓	[52]

S. no.	Food component added	Added to starch	Impact on starch digestibility	Reference
22.	Alfalfa seed (15%, 30%, 45%)	Rice flour	↓	[100]
23.	Hydrolyzed protein (12%)	Wheat flour	↓	[13]
24.	Gluten (20%)	Wheat starch	↓	[101]
25.	Whey protein isolate (2.5%, 4.5%, 10%)	Native corn starch	↓	[102]
26.	Rice globulin (2.5%)	Rice flour	No effect on digestibility	[103]
27.	Soybean peptide (5%, 10%, 15%)	Corn or potato starch	↓	[104]
28.	Chickpea protein (8%)	Rice flour	↓	[105]
Fiber				
29.	Oat fiber (10%)	GF bread	↓	[70]
30.	Oat fiber (>5%)	Pasta	↓	[106]
31.	Inulin (12%)	GF bread	↓	[107]
32.	Cellulose (50%)	Potato starch	↓	[79]
33.	Cellulose (9–83%)	Maize starch	↓	[66]
34.	<i>Lentinus</i> β-glucan (20%)	Wheat flour	↓	[73]
35.	Glucomannan (0.1–0.2%)	Rice starch	↓	[70]
36.	Xanthun gum (0.4%)	Rice starch	↓	[71]
37.	RSIV (Novelose 480) (10%)	Pasta	↓	[108]
38.	RSIV (Novelose 480) (10%)	GF bread	↓	[69]
39.	RSII (Native HA maize starch) (20%)	GF bread	↓	[109]
40.	RSII (Hi-maize [™] 260) (20%)	Pasta	↓	[106]
41.	RSII (Fibersym [™] 70) (20%)	Pasta	↓	[77]
42.	Okara (10%)	Rice noodles	↓	[74]

Table 1.
 List of various food components added to starch and their effect on starch digestibility.

2. Conclusion

Binary interactions among the nutrient types and starch mediate the glycemic amplitude of real food systems. Among the binary interactions (starch-lipid, starch-protein, starch-fiber), the role has been extensively characterized in limiting the enzyme penetrance, altering the molecular configuration, starch digestibility, and thus in turn GR. Understanding such binary interactions, not only shares a logical explanation for the low GI of whole-grain foods but also the immense role of such cereals in diabetic-friendly foods. Even though the existing rationale supports the fact that multiple food matrix interaction studies at a time are difficult, it's indeed vital to study ternary (three-way) and quaternary (four-way) interactions and their

role in limiting the glycemic response. Finally, it's important to keep in mind that altering starch's nutritional qualities can also change its desired physicochemical and sensory qualities, affecting food quality that should be considered while developing novel foods.

Conflict of interest

The authors declare no conflict of interest.

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