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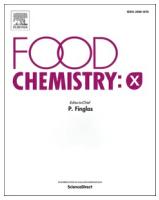

Functional foods with a tailored glycemic response based on food matrix and its interactions: Can it be a reality?

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ABSTRACT

Functional foods are considered the future of nutrition because they benefit human health and environmental sustainability. They offer natural solutions for managing post-prandial glycemia and its long-term consequences. Therefore, understanding the composition and inherent dynamics of the functional food matrix (FM) is crucial. Within the FM, components like proteins, fats, carbohydrates, phenolic compounds, fibres, and minor elements interact dynamically, highlighting how individual components within the system behave. This review highlights the significance of diverse FM interactions in modulating inherent glycemic potential (IGP). These interactions comprise major binary, ternary, quaternary interactions, and minor interactions, in contemporary functional food formulations that include starch-derived additives, biopeptides, and flavouring agents. The starch quality matrix (SQM), a prediction model for customised functional foods with low IGP, has been briefed as a pilot concept. We also investigate the impact of these interactions on gut health, fill in the knowledge gaps, and provide recommendations for further study.

1. Introduction

Human health comprises a unique biological, behavioral, and environmental combination. The functional food sector was driven by the sharp rise in consumer interest in the potential of certain foods and their bioactive components to improve health. Through Ayurveda, Ying and Yang, and other practices, the holistic impact of food on health has been ingrained in nations and customs around the globe. Many more, functional foods like millets and mushrooms have been linked to lower CO_2 footprints, improved soil and water quality, and biodiversity conservation (Granato, Zabetakis and Koidis, 2023). Many more epidemiological studies clearly show the effect of functional foods in alleviating the major threatening chronic metabolic diseases like type II diabetes mellitus (T2DM), obesity, chronic vascular disease (CVD) and so on. But major limitation of such studies was their focus on single food matrix (FM) component and now it's very well evident that every component as well as their dynamics plays a significant role. More than their presence in the physical matrix, their interactions ultimately govern our health and the reason behind how they uniquely manifest such health attributes (Granato et al., 2023).

This understanding has led to the concept of 'FM' defined by the USDA as the relationship between nutrient and non-nutrient components in food, including their molecular relationship, such as chemical bonds (USDA NAL Glossary, 2015). Furthermore, the interactions in the FM significantly affect the release, stability, accessibility, mass transfer, and bioavailability of nutrients in whole foods (Güler & Sensoy, 2023). However, foods like meat, grain, legumes, milk, mushrooms, and bread have diverse dietary structures, resulting in various digestion and bioaccessibility patterns (Fardet and Rock, 2022). As a result, more comprehensive insights are required to understand how the FM changes from harvesting through digestion to develop functionally better meals with more bioactive components targeting health signatures in human.

Carbohydrate, a major food nutrient, has gained sufficient attention in this discussion. Researchers have classified carbohydrates as 'simple' or 'complex' based on the human body's polymerization rates and bioavailability patterns. Various indices, such as glycemic load (GL), glycemic index (GI), and glycemic response (GR), are used to rank carbohydrates in foods and assess their impact on post - prandial glucose

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release in the bloodstream. GR refers to changes in blood glucose levels following the consumption of any meal, whereas GI quantifies meals based on their post - prandial glucose release (Kim & Je, 2023). It is calculated as the ratio of the area under the curve (AUC) of GR after food consumption to the AUC of GR after consuming a reference sample, typically glucose (Wang, Li, Peng, Liu and Yu, 2023). However, GL is another parameter used here to classify carbohydrate-rich. Walter Willett and colleagues introduced the GL concept in 1997 (https://care.diabetesjournals.org), which has recently been updated (Dong, Eustis and Hawkins, 2023). However, it considers the concept of GI and the total available carbohydrates in a carbohydrate-rich diet (GL = GI × total available carbohydrates). Earlier reports provided a formula for evaluating the GI of an entire diet by incorporating the GI of each meal consumed by an individual, *i.e.,* Meal GI=.

$$\frac{(\{[\text{GI Food A} \times \text{g available carbohydrate (avail CHO) Food A}] + [\text{GIFood B} \times \text{g available carbohydrate (avail CHO) Food B} + \ldots..]\})}{(\text{total g of available carbohydrate})} \quad 1$$

(Chekima et al., 2022; Dodd, Williams, Brown, & Venn, 2011). It can be estimated using either the predicted (G_{pred}) or adjusted (G_{adj}) formula where G_{pred} represents the carbohydrate content of the meal, and G_{adj} includes fats or proteins present in addition to carbohydrates during meal estimation (Wolever, 2013). However, studies have revealed inconsistent results with these two crucial indices. Some researchers found that G_{pred} overestimated the actual GI value of a meal (Dodd et al., 2011; Östman, Granfeldt, Persson and Björck, 2005), while others observed that both G_{pred} and G_{adj} were effective in calculating the total GI of a meal (Chekima et al., 2022). Therefore, several parameters are needed to analyze the impact of glucose on post - prandial metabolism, depending on carbohydrate choices and availability.

In this context, a new term, "Inherent Glycemic Potential (IGP)," was introduced to assess *in vitro* starch bioavailability using an in-house oro - gastro - intestinal digestion method in food matrices like crops (Krishnan et al., 2021). It comprehends how any crop responds intrinsically to glucose release, how the intrinsic parameters present along with starch in that crop react during digestion, how other exogenous factors impact its glucose-releasing potential, and so on. Although there are differences in indices, *in vitro* assays are preferred because they provide a quicker and less expensive window than *in vivo* techniques to determine the rate and amount of glucose digestion. (Table 1). However, according to starch digestibility patterns or *in-vitro* amylolysis rates, rapid digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) have huge significance in this context of GR or IGP. As per their name indicates, these starch types differ in their time to reach and digest in small intestine. Even though all types have respective contribution in GR, RS plays a pivotal role not only just for digestion rather for remaining hydrolyzation in small intestine and fermentation process. This type of non-digestible, highly fermentable starch is classified as dietary fibre (DF) because of its similar physiological properties on humans. For example, like DFs, RS can slowly affect the rate of gastric emptying. During mobility in gastrointestinal tract, RS produces short-chain fatty acids (SCFA) in the large intestine by which it executes beneficial effects on glucose metabolism (Kim, Park, & Kim, 2024). Consumption of RS may also reduce GR of subsequent meals as compared to RDS. The reason behind such phenomena could be enhanced insulin secretion which leads to glucose dependent insulinotropic polypeptide (GIP). Nonetheless, SCFA produced during RS fermentation showed profound effects on glucose homeostasis in liver, muscle tissues and induces gut hormones release, such as glucagon like peptide (GLP-1) and peptide YY (PYY) which, in turn, add noteworthy aspect to the human glucose homeostasis. As per American Diabetes Association, the most important determinant of post-prandial GR is the carbohydrate quantity while quality which refers to the rate of digestion also alters the post meal glucose levels. Quantity relates to the total available carbohydrates comprising monomeric sugars and polymeric starch in the matrix. At the same time, quality involves the extent of starch fraction digestion in two dimensions - time (such as rapidly or slowly digestible fractions) and location (distal and proximal small intestine digestion). The intricate interplay can trigger a natural feedback loop, inducing satiety by activating the gut-brain axis (Lim, Ferruzzi and Hamaker, 2022). Thus, to elucidate further on the concepts of nutrient bioavailability, understanding the interactions among FM components is of prime importance.

2. Significance of FM interactions: Relevance in glycemic control

Every food possesses unique structural, physical, and chemical characteristics that affect the digestion, absorption, and metabolic processes in the body. Therefore, to grasp the fundamental concept behind each food structure and formulation, a thorough understanding of how the components within the FM interact through physical and chemical reactions is required. Despite these reactions being governed by thermodynamics and kinetics, achieving a complete mechanistic understanding of how the FM affects nutrient bioavailability remains challenging (Sarpong, Dwumfour, Rashid and Aly, 2022). Furthermore, the complexity arises from the observation that the same chemical reactions can form different outcomes in natural foods compared to simple aqueous reactions (Han et al., 2022). This disparity occurs because food is not an ideal solution; its behavior varies depending on its physical state and context. In the light of controlling hyperglycemia, the term "FM interactions' has emerged where matrix components interact with each other to perform their functions through properties such as diffusivity, strength, stability, and scaffolding (Fig. 1). Existing research has emphasized that interactions within the FM distinctly affect taste, texture, flavour perception and physiological functions. For example, the FM breaks down during chewing, leading to specific interactions with salivary enzymes that influence taste perception. For instance, solid foods containing fibre or protein are more satiating than liquid foods (Akhlaghi, 2022). Similarly, foods containing gum or gelling fibres promote fullness by postponing the emptying and mass transfer of the stomach. This, in turn, slows down the enzymatic processes and/or the distension of the stomach antrum because of their high viscosity (McRorie Jr and McKeown, 2017). Soybean, well known functional food was used as the model to prove that the effect of bioactives like isoflavones immensely vary across different stages of human life. Isoflavones alone and in combination with other matrix components like lignans or proteins have also reported with varied functionality towards bone health (Jeffery, 2005). To elucidate the fundamental science behind matrix interactions and nutrient availability, nutritional researchers have categorized FM into various facets, such as binary, ternary, and quaternary interactions, based on their components. The essential interactions among matrix components ultimately influence various aspects of food products, such as gelatinization, retrogradation, sensory and cooking qualities, swelling capacities, rheological properties (like viscosity, pasting, setback, breakdown), textural properties, and nutritional qualities (including glucose, protein, and lipid bioavailability), as well as bio-accessibility, digestibility, and glycemic

Table 1
Major *in vitro* starch hydrolysis methods with their respective benefits and limitations.

In-vitro Methods	Equation	Procedure	Benefits	Limitations	References
Englyst methods	TS = (TG-FG) *0.9 RDS = (G20 − FG) *0.9 SDS = (G120 − G20) *0.9 RS = TS − RDS − SDS	***Oral phase:*** The chewing process has been simulated through mincers (plate 0.9 cm) ***Gastric Phase:*** This process did not include pepsin proteolytic step. ***Intestinal Phase:*** Minced food samples in screw-top tubes are mixed with enzymes (pancreatin, amyloglucosidase and invertase), followed by shaking in a water bath at pH 5.2 and 37 °C over 2 h.	1. It includes the measurement of free glucose (FG) and total glucose (TG) in order to accurately calculate each fraction. 2. Various types of resistant starch (RS) RS1, RS2 and retrograded RS3 can also be measured based on this procedure. 3. This method includes only two timepoint starch bioavailability analysis (20 min & 120 min) without overall digestion mechanism.	1. It does not incorporate digestion process in the gastric phase (No pepsin proteolytic step). 2. This process is somewhat complicated as compared to other starch bioavailability measurements.	Englyst, Kingman and Cummings 1992.
Goñi's Method	$C = C\infty\ (1 - e^{kt})$ C: concentration of hydrolyzed starch C∞: equilibrium concentration of hydrolyzed starch k: kinetic constant	***Oral Phase:*** The sample food is homogenized. ***Gastric Phase:*** Proteolysis step is included here by pepsin at pH 1.5 40 °C for 1 h. ***Intestinal Phase:*** The sample kept in an intestinal phase incubation pH of 6.9 with addition of Tris-Maleate buffer. Samples were then digested by α-amylase in a shaking water bath (37 °C) with 1 mL aliquots withdrawn every 30 min during a 3 h digestion. These aliquots were further digested using amyloglucosidase into glucose at 60 °C shaking water bath.	1. Hydrolysis percentage is calculated based on resistant starch (RS) and digestible starch (DS) content; 2. It is somewhat simpler than the Englyst method. 3. It is pseudo first order reaction where k is a function of the fixed amylase and substrate concentrations during starch digestion.	1. This method requires pretreatment to remove lipids and protein, and additional analysis is needed to measure the RS content. 2. The reaction parameters of this equation are different for different foods, and need to be calculated individually for each sample. 3. When complexity of starch digestion is considered, estimation through this equation is not suitable at that moment.	Goñi, Garcia-Alonso and Saura-Calixto,1997.
Guraya's Method	$\%\ RDS = \frac{D-E}{F} \times 100$ D = mg maltose produced on digestion at 1 h E = mg maltose at 0 h digestion F = total starch (mg) $\%\ SDS = \frac{G-H}{F} \times 100$ G = maximum mg maltose produced on digestion when no further increase is noticed H = mg maltose at 1 h digestion F = total starch (mg) $\%\ RS = \frac{F-G}{F} \times 100$ G = maximum mg maltose produced on digestion when no further increase is noticed F = total starch (mg)	No separate oral, gastric, intestinal phase has taken for consideration in this method.	1. This is the simplest method to measure RDS, SDS, and RS based on the measurement of the maltose produced by porcine pancreatic α-amylase hydrolysis (1% *w/v*, phosphate buffer pH 6.9). 2. The maltose concentration is measured using the 3,5-dinitrosalicylic acid (DNS) assay.	1. This method cannot be used to measure digestion fractions of some glucose-containing polymers, like pullulan that is resistant to α-amylase with its special structure, that have a SDS property. 2. Additionally, using maltose as the standard to represent starch fractions is not scientifically accurate as the maltose is only a small portion of the hydrolysis products from α-amylase digestion.	Guraya, Jame, and Champagne, 2001.
Standardized Static Digestion Method	Starch hydrolysis = $\frac{Sh}{Si} = \frac{0.9 \times Gh}{Si}$ Here, Sh = Amount of starch hydrolyzed, Si = Initial starch in the Samples Gh = Amount of glucose produced from the sample	***Oral Phase:*** Simulated Salivary Fluid (SSF) electrolyte stock solution (7.0 mL, pH 7) was mixed with cooked starch samples. 1 mL of the salivary α-amylase solution of 1500 U/mL was added followed by 50 μL of 0.3 M $CaCl_2$ and 50 μL of water. The mixture was thoroughly mixed and incubated for 2 min at 37 °C at 150 rpm in a water bath. ***Gastric phase:*** The oral mixture was mixed with 18 mL of the simulated gastric fluid (SGF) electrolyte stock solution and 10 μL of 0.3 M $CaCl_2$ followed by adjusting pH to 3.0 with 3 M HCl. 1.5 mL	1. The reducing sugar released from samples was determined using 3, 5-dinitrosalicylic acid (DNS) method. Each digestion process can be analyzed by taking absorbance at each phase.	1. No such limitations have been reported till date.	Minekus et al., 2014.

(*continued on next page*)

Table 1 (*continued*)

In-vitro Methods	Equation	Procedure	Benefits	Limitations	References
		of porcine pepsin stock solution (2000 U/mL in the final mixture) was added and the volume was adjusted to 40 mL with water. The sample was incubated for 120 min at 37 °C at 150 rpm. ***Intestinal Phase:*** The resulted gastric digesta was mixed with 22 mL of simulated intestinal fluid (SIF) electrolyte stock solution and pH was adjusted to 7.0 with 1 M NaOH. 80 μL of 0.3 MCaCl2 and 5 mL of fresh bile (160 mM in fresh bile) were added to the mixture. 10 mL of a pancreatin solution made up in the SIF electrolyte stock solution was added to reach 100 U/mL of trypsin activity in the final mixture. Final volume was made up with water to 80 mL. The sample was incubated in a water bath for 120 min under controlled conditions (37 °C, 150 rpm). The mixture was then agitated at waterbath for 120 min at 37 °C.			
Jenkins Method	SO = [F + DJ] - [D J] Here, where SO denotes sugars and oligosaccharides, [F + DJ] and [DJ] are the concentrations of sugars and oligosaccharides surrounding the dialysis bags containing either food, F, and digestive juice, DJ, or digestive juice alone. Besides, the enzymatically liberated component SOE was estimated by: $SO_E = [F + DJ]-[F + DJ_B]$ where DJ_B is boiled digestive juice.	***Oral Phase:*** Carbohydrates (2 g) were boiled in a minimum of water with 2 g salt and ground to a smooth paste in a pestle and mortar. Then it was mixed separately with 2.5 mL of fresh pooled human saliva. ***Intestinal phase:*** 7.5 mL of pooled human post-prandial jejunal juice obtained from individuals with normal pancreatic function was added into this. Finally, volume of each food-enzyme mixture was the same, the volumes were adjusted to 30 mL by addition of distilled water.	1. Results are given for free glucose and total sugars and oligosaccharides, *i.e.,* maltose, maltotriose and dextrins, measured as glucose after acid hydrolysis, which were liberated during the digestion of the foods *in vitro*.	Enzyme in gastric phase has not included in this method. Further tests were needed with all foods using saliva and jejunal juice after inactivation of the digestive enzymes by boiling so that allowance could be made for free sugars already in the foods.	Jenkins et al., 1982.
Edward's method	Starch amylolysis (%) = $\frac{[\text{maltose}]t}{[\text{maltose}]\text{substrate}} \times 100$ Where [maltose]t is the maltose equivalent concentration (after baseline correction) measured in the liquid phase of the reaction mixture at a time point t, and [maltose]substrate is the theoretical maltose equivalent concentration, assuming that all starch within the food sample can be converted to maltose.	Freshly prepared food materials were weighed and suspended in 10 mL phosphate buffered saline (PBS, pH 7.4). Sample tubes were mixed for 20 min at 37 °C on a rotary mixer (20 rpm, 30° angle) inside an incubator to equilibrate. After that, porcine pancreatic α-amylase were added to achieve an activity of 4 U/mL in the digestion mixture (*i.e.*, containing 10 mg mL^{-1} starch). Tubes were returned to the mixer in the incubator after addition of amylase and incubated at 37 °C in the mixer for the duration of the digestion.	1. Starch-amylase ratio has been kept constant for all food products tested such that any differences observed reflect the starch digestibility of the food rather than their variable starch contents.	1. In this method, the conversion assumes that all amylolysis of starch produces maltose, and this approach does not precisely account for minor products of starch amylolysis (glucose, α- dextrins and maltodextrins) thereby resulting in a net underestimation of total starch amylolysis	Edwards, Cochetel, Setterfield, Perez-Moral and Warren, 2019.

potential. Notably, the specific molecular arrangements formed through these macro-molecular interactions significantly impact the development of functional food formulations in the food industry. However, there is currently limited information available on this subject matter. Therefore, this review aims to comprehensively explore multiple facets of FM interactions and address the current knowledge gaps to gain a more precise and deeper understanding of glucose bioavailability for managing hyperglycemia and promoting human well-being.

Several clinical trials have demonstrated the impact of FM interactions on glycemic control. For example, a study involving healthy

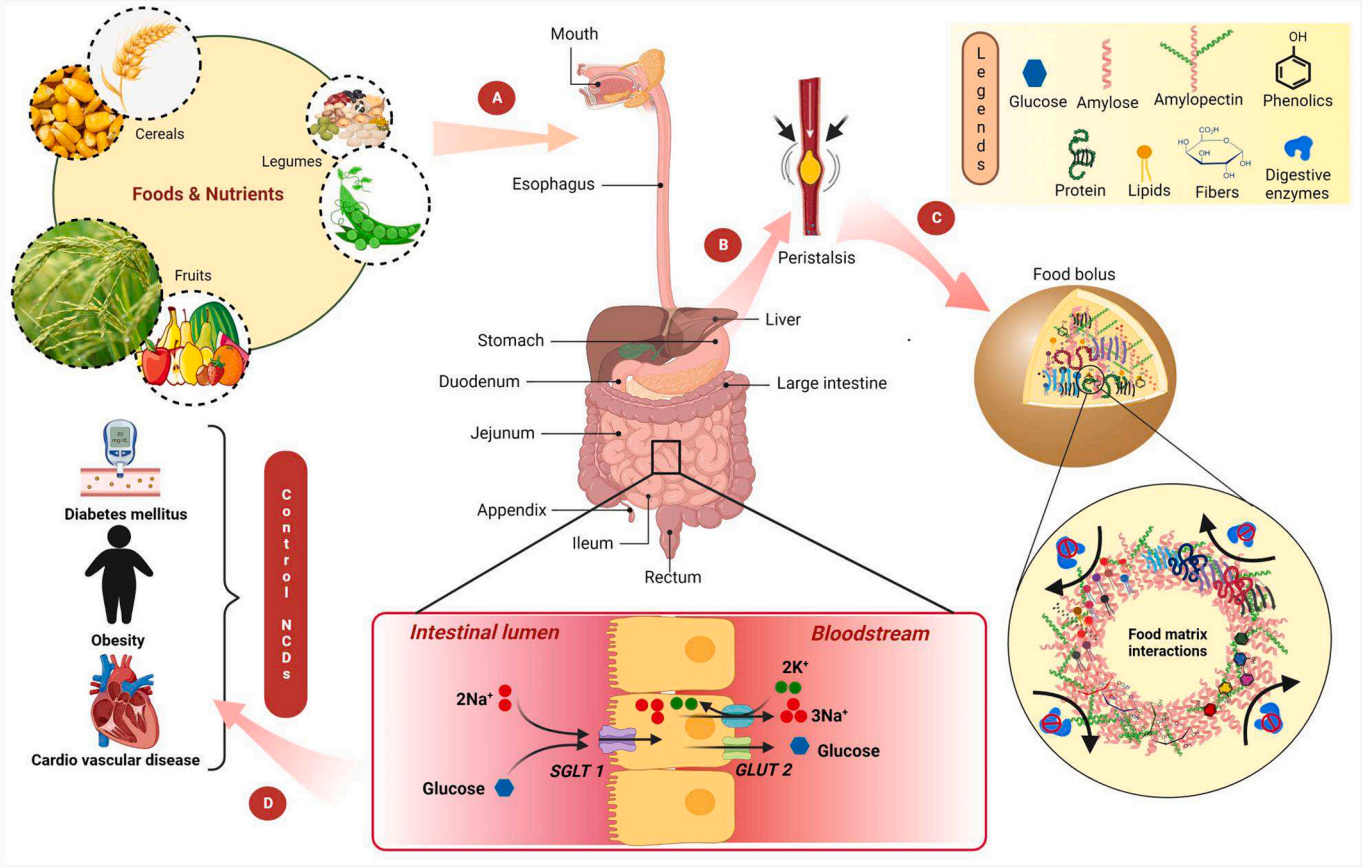

Fig. 1. Schematic representation of how diverse food matrix components interact and are absorbed in the human body with a focus on limiting the glycemic response. A) Different types of starch-based foods were ingested into the body; B) Peristaltic-like automatic wave-motion begins on the throat and moves food through the gastrointestinal tract; C) During movement, food bolus comprises of various matrix interactions among starch, lipid, protein, fibre, phenolics. D) Diverse scale of food matrix interactions (binary, ternary, quaternary) ultimately impedes starch degradation and can control glucose release as well as non-communicable diseases (NCDs). [Created with BioRender.com].

overweight adults found that daily consumption of 30 g of high amylose maize-RS 2 (HAM-RS2) for six weeks improved glucose regulation and modulated plasma biomarkers like GLP - 1, PYY and leptin without affecting body composition (Maziarz et al., 2017). Similarly, Wang et al., (2023), Wang et al. (2023) recently demonstrated that consuming heat-treated foxtail millet starch and protein led to reductions in fasting blood glucose and insulin levels. The results suggest that heat-moisture treatment of foxtail millet increases RS and SDS content, contributing to a hypoglycemic effect by resisting digestion in the small intestine. These findings emphasize that naturally encapsulated foods' tissue or cellular structures remain intact during mastication, gastro-ileal transit, and gastro-colonic digestive processes, allowing nutrients to be absorbed to varying degrees. Therefore, recognizing the impact of FM on nutrient bioavailability, both qualitative and quantitative assessment of food microstructures, becomes relevant. Additionally, by utilising varied meals and food formulations, postprandial GR has been improved. Glycemia has been shown to change when foods with distinct nutritional profiles are consumed together. In particular, the so-called stomach emptying is delayed when foods high in fibre, fat, and/or protein, complex carbohydrates are present alone or in combination with them, as well as when foods high in carbs are combined with these (Slavin, 2013). Eating the same amount of carbohydrates in the form of white rice or in the form of white rice mixed with beans or chickpeas (mixed meal) was shown to attenuate GR compared to rice alone (Kaur, Ranawana and Henry, 2016). Thus, the dynamics with in food matrices are critical.

3. FM and its interactions

Foods typically comprise matrix components (nutrients) such as proteins, fats, carbohydrates, phenolic compounds, fibres, and some minor elements such as vitamins, and minerals. The interplay between these matrix components can result in various effects, including additive, synergistic, antagonistic, masking, and neutralization within food formulations (Pan et al., 2018). Recent advancements in food - omics technology have highlighted the complex and intricate relationships among matrix components, which profoundly impact nutritional bioefficacy positively or negatively. An additive effect occurs when the combined effects of two or more components equal the sum of the effects of each component acting independently. Conversely, a synergistic effect arises when individual matrix components interact, leading to an overall effect greater than the sum of their individual effects (Zhang et al., 2020). On the contrary, antagonism occurs when matrix components combine to produce an effect that is less than the sum of their individual effects (Pan et al., 2018). Masking or neutralizing effects occur when simultaneous interactions among matrix components either reduce the overall effect or have no effect on the food formulation (Sęczyk, Gawlik-Dziki and Świeca, 2021, Tomar et al., 2022). Although the understanding of FM interactions through the application of these effects may be lacking in various food industries, it is well - established that different hierarchical levels of food interactions exist, including binary, ternary, and quaternary interactions (Fig. 2).

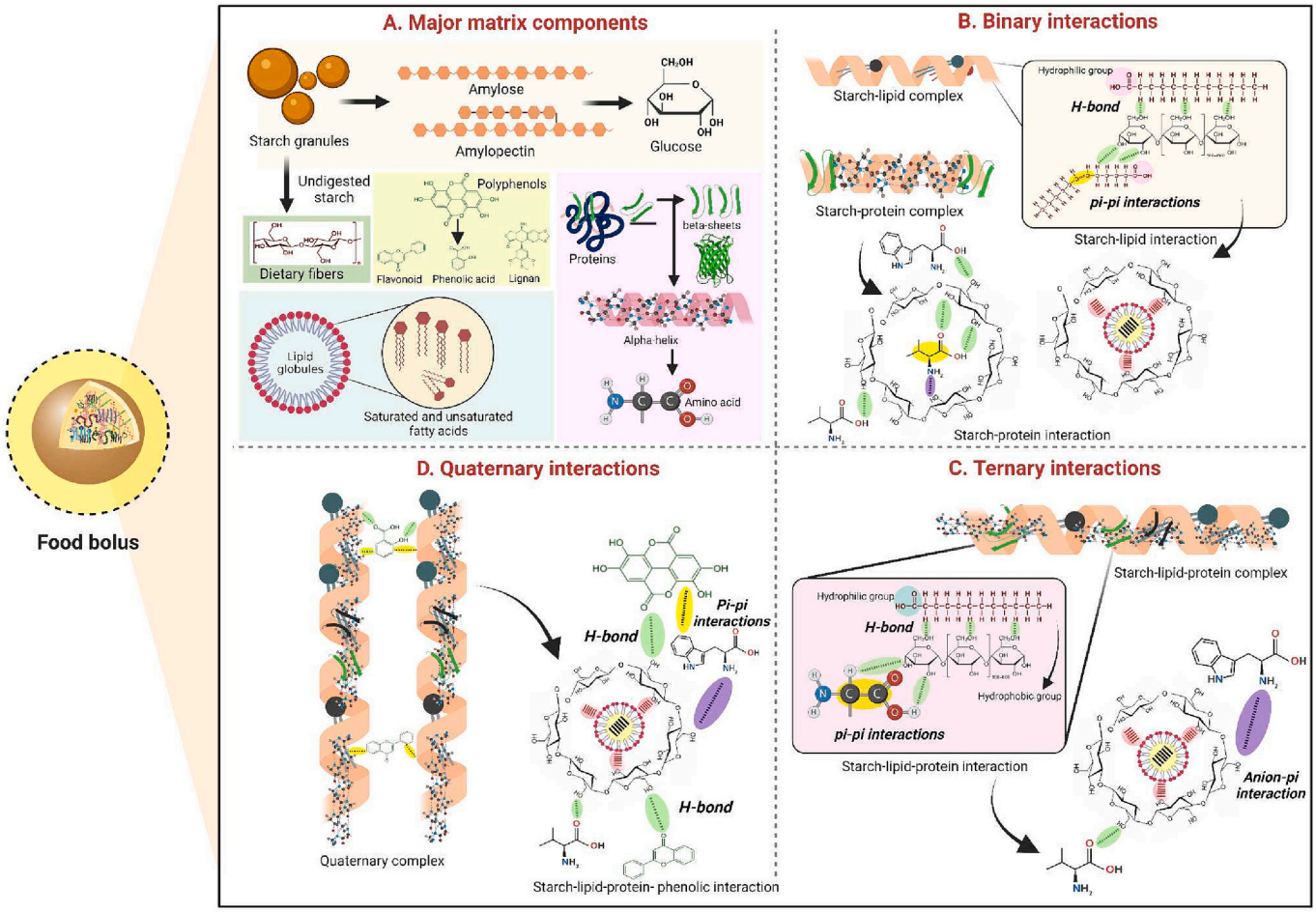

Fig. 2. Prevalent food matrix interactions in starch-based foods. Food matrix of functional foods comprises of different dimensional molecular interactions. Especially, in starch-based foods, major matrix components (A) and its interactions have been categorized into three layers like (B) binary (two components' interactions), (C) ternary (three components' interactions), and (D) quaternary (four components' interactions) depending on matrix components. These vital interactions ultimately govern controlled release of glucose and regulate starch-glucose homeostasis in the body. The hydrophobic, electrostatic interactions governed among starch, lipid, protein and phenolics molecules were mentioned in the diagram. For ease of reading, only limited matrix interactions have represented through different colors in the figure and cyclic representation of starch represents its transversal view. [Created with BioRender.com].

3.1. *Binary interactions*

Binary interactions among starch, lipid, protein, and phenolic compounds are extensively characterized as FM events involving various *in vitro / in vivo* component addition or depletion strategies (Krishnan et al., 2020, Krishnan et al., 2022a, 2022b). These components undergo multiple changes and modifications during processing, affecting their functional quality, flavour, shelf-life texture, and other properties. Thus, we discuss four significant binary interactions: starch-lipid (S - L), starch-protein (S - P), starch-fibre (S - F), and starch-phenolics (S - Ph) and its role in regulating IGP. Considering newer functional food formulations, it's important to consider other possible interacting components like biopeptides, flavouring agents, starch-derived additives and their role in tailoring glycemia.

3.1.1. *S -L interactions*

Since the ultimate IGP depends on the abundance and availability of starch and the types of matrix interactions, various starch-based binary interactions have been comprehensively characterized. Among these, S - L interactions provide essential insights into starch bioavailability and IGP. However, various lipids such as phospholipids, glycerol, and free fatty acids in the existing food system have also contributed remarkably to energy sources, membrane structure, and functions. Being inherently helical, starch can include lipid molecules into the lumen of its structure to form stable inclusion complexes. Studies have referred to this type of complex as RS-V type structure, which mainly resists the action of digestive enzymes and lowers glucose release (Putseys, Derde, Lamberts, Ostman and BJOrck, I. M., & Delcour, J. A., 2010; Krishnan et al., 2020). It is essential to investigate a few fundamental properties of this inclusion complex to understand the biochemical events of glucose release during this complexation. Some studies have suggested that these structures can exist intrinsically in the FM and be present extrinsically through several exogenous applications (Mondal et al., 2022). Various covalent and non-covalent interactions, such as hydrogen bonds, electrostatic interactions, hydrophobic interactions, and van der Waals interactions, have been found to play a superior role during these interactions. However, this structure can accommodate a maximum of six glucosyl residues per turn with linear lipids (Feng et al., 2018). In contrast, in the case of other lipids (larger size than linear lipids), seven or eight glycosyl molecules can also be present (Obiro, Sinha Ray and Emmambux, 2012). Considering the impacts of the S - L complex on IGP (Krishnan et al., 2020), few key factors, such as amylose content, amylopectin structure, fatty acid chain length, and degree of saturation, warrant further discussion.

Studies over the decades suggested that amylose can easily interact with lipid molecules and form binary complexes (Guan, Zhao and Thaiudom, 2022). Other studies have highlighted the significant contribution of amylopectin chain length to this interaction (Wang et al.

2023). Both components of starch molecules (amylose and amylopectin) form the stable S - L structure through the hydrophilic –OH groups on the outer side and hydrophobic glycosidic bonds in the inner cavity, influencing FM interactions. The fatty acid chain length further supplements this concept *via* various interactions. For example, the IGP of a starchy diet depends on the glucose released from the food, which, in turn, relies on the strength of the complex formed between starch and lipid molecules. Qin et al. (2019) suggested that a fatty acid chain length of C14 can form stable crystalline complexes, while others have explained that chain lengths of C16 or C18 contribute to the type II crystalline complexation in differential scanning calorimetry (DSC) thermograms with lipids (Tufvesson, Wahlgren, & Eliasson, 2003a,b). Therefore, it is evident from the studies mentioned above that the chain length of fatty acids significantly contributes to the strength of the S - L complex. Further, saturated fatty acids (SFA) have also been implicated as vital in RS - V complex formation. A recent study by Guo, Hou, Liu, Chen and Zheng (2021) highlighted the role of unsaturated fatty acids (UFA) in the formation of the S - L complex, explaining a new dimension where the double bonds of UFA form a bent-like configuration, reducing the number of carbon atoms available for complexation. These conformational changes enhance the complexity of starch molecules compared to SFA. Validation studies were carried out in different food matrices incorporating components with varied fat profiles. Incorporating two types of high-fat sauces (pesto and tomato sauce with extra virgin olive oil) into a pasta dish and a rice dish, revealed that white rice showed an increase in AUC compared to pasta (2236.84 ± 217.92 mg min/dl and 1478.62 ± 232.33 mg min/dl, respectively) that was reduced depending on the sauce used (rice + tomato sauce with olive oil = 1509.24 ± 185.5 mg min/dl; rice + pesto = 1395.78 ± 87.3 mg min/dl). Adding these sauces on pasta dishes, it was observed a major reduction by adding tomato sauce compared to pesto (pasta + tomato sauce with olive oil = 920.31 ± 124.27 mg min/dl; pasta + pesto = 1055.39 ± 189.11 mg min/dl) (Chiavaroli et al., 2021). Such S-L inclusion complex has also been functionally found to reduce the solubility, swelling power, retrogradation, and gel rigidity of starch, increase the gelatinization temperature, and inhibit enzymatic hydrolysis after interacting with lipid and water molecules in system. Consequently, this binary interaction (S - L) has a significant impact on lowering glucose release and IGP.

Similarly, mounting recent evidences were also lined up in literature to highlight the significance of S – L complex in GR. In this context, Liu et al. (2024) proposed a strategy of extrusion-debranching-complexing to enhance the yield of RS - V for industrial production of amylose-lipid complex. The double extrusion process of enzymatically debranched extruded corn starch-lauric acid provides higher thermal stability with 105–145 $^{\circ}C$ dissociation temperatures, improved RS - V contents >30% which in turn reduces the GR for industrial applications. However, another study on influence of different fats/oil types (varying degree of saturation and chain length) on baking bread suggested that the concept of saturated fatty acids *i.e.,* lauric acid and myristic acid present in coconut oil lowers GR of baked products which help further in the management of chronic diseases. Krishnan et al. (2020) also proposed similar thoughts where complexing ability and RS formation were influenced by chain length and degrees of saturation of fatty acids in rice varieties. However, beside this, this study also highlighted the establishment of a quaternary structure between starch, lipid, protein, and phenolics, which finally resolves the enhanced RS - V contents and decreased glycemic potential as well. Overall, the above-mentioned studies suggested that binary FM interactions between starch and lipid molecules are critical for developing functional meals with low GR.

3.1.2. S – P interactions

Protein (endogenous or exogenous) is the second-highest food component and significantly regulates IGP. However, earlier studies reported that starch and proteins could not be thermodynamically compatible during complex formation (Jamilah et al., 2009). Depending on pH and other factors in the FM, acidic or basic amino acids, proteins interact with a starch molecule, confirming that a stable native protein structure is vital for this complex. In addition, the S - P interplay has a significant impact on IGP as well as the glycemic profile of foods. An interesting correlation has been found between starch and protein, indicating that increasing protein content in starchy foods reduces insulinemic responses and IGP. Previous studies by Ye, Hu, Luo, McClements, Liang & Liu, (2018) suggested that endogenously present protein creates a barrier with starch granules, reducing enzyme accessibility and releasing glucose molecules. However, other researchers hypothesized that exogenous proteins encapsulate starch granules, thereby minimizing contact and the amylolysis process (Zhang et al., 2023). Similarly, another report by Chi, Li, Zhang, Chen, Li & Wang, (2017) explained that the non-covalent binding of protein with starch molecules reduces the starch hydrolysis as a catalytic substrate, while Lu, Donner, Yada and Liu (2016) unraveled a non-catalytic method of protein binding with starch that ultimately prevents catalytic amylose from binding to monomeric glucose molecules.

Therefore, various depletion studies of protein have been performed to explore this type of binary interaction (S - P) (Li et al., 2023; Ye et al., 2018). One study applied this concept to wheat products through *in vivo* and *in vitro* approaches, where meals of white bread formulated using regular or gluten-free flour were given to healthy individuals. The result suggested a substantial elevation of blood glucose levels after consuming bread formulated with gluten-free flour; this was further confirmed through the *in vitro* approach. Later, glucose release was considerably reduced when wheat gluten was reintroduced to the gluten-free flour. Hence, the possible mechanism behind such results could be the presence of wheat flour (or matrix interplay), where starch molecules were enclosed by a protein network that restricted the rate of hydrolysis in the small intestine lumen. This confirms that protein molecules create a matrix-like network surrounding the starch molecules, ultimately resisting blood glucose release. Studies have concluded that consumption of foods rich in carbohydrate together with protein (about 25 g) of animal origin (meat, fish, eggs, dairy products, and derivatives) or vegetal origin (legumes, nuts, and seeds) attenuates GR. In addition, various exogenous factors like thermal/mechanical processing have been found to alter the level of S - P interaction, influencing the overall IGP. Furthermore, Parada and Aguilera (2011) reported that "appropriate" kneading/mixing influences protein matrix (gluten) formation *via* disulfide linkages, while extreme kneading/mixing leads to breakage of S - P lineages and strength, thus promoting IGP through enhancing digestion.

Functional food industry has also embraced food-derived bioactive peptides and protein hydrolysates due to their proven health benefits. These short amino acid sequences of food proteins released with endogenous or exogenous or microbial protease activity have reported to interact with starch. It has been shown that such cryptides from different sources (milk, egg, fish, pulses, legumes, and cereals) have antidiabetic potential. These peptides assist in lowering blood glucose levels, enhance insulin absorption, and inhibit important enzymes that contribute to the onset and progression of diabetes. It has been proposed that bioactive peptides in camel milk protein hydrolysates prevent intestinal α-glucosidases from assisting in the digestion of starch (Althnaibat, Bruce & Gänzle, 2023). Human *in vivo* trials have also shown the effects of hydrolysates of milk protein (Sartorius et al., 2019), casein (Geerts et al., 2011), and whey protein (Chen et al., 2020) on IGP; however, they have not been able to identify specific peptides that have inhibitory activity on starch digestion. It has been shown that cryptides can easily react chemically at their nucleophilic amino, carboxy, imino and sulhydryl groups. As the precise interaction of cryptides with starch depends on their amino acid composition, sequence and length, more comprehensive studies are required.

3.1.3. S - F interactions

Various researchers have studied the importance of DF in human

health and its role in regulating IGP over decades. The understanding and definition of DF have been modified since 1950. Codex Alimentarius Commission (CAC) has recently defined DF as ten or more monomeric units of carbohydrate polymers, which are not easily hydrolyzable by the endogenous digestive enzymes in the gastrointestinal tract of humans (CAC, 2009). Consequently, based on solubility, DF has been classified into two categories: soluble digestible fibre (SDF) and insoluble digestible fibre (IDF). Interestingly, though, among the two types of DF, SDF (β – glucan) has been found to have a great role in lowering IGP compared to IDF (Krishnan et al., 2007). A previous report by Wu, Qiao, Tian, Tan and Fang (2021) has described how in the FM, starch and fibre interact to form a dense matrix surrounding starch molecules, acting as an effective barrier against digestive enzymes and thereby reducing IGP. Studies have shown that fibres do not react with starch during the extrusion process. Thus, to improve surface interactions with starch molecules, fibres are chemically modified using alkaline, peroxide, and acetylation, removing polyphenols, hemicelluloses, and pectin substances while enhancing their functional properties (Dey et al., 2021). Similarly, Hao et al. (2023) explained that oat β–glucan as well as starch were used to minimize enzyme dispersal by ball milling treatment and, however, the rate of starch hydrolysis, resulting in a slower glucose release with improved molecular configuration. Furthermore, Sciarini et al. (2017) highlighted that adding 5% Inulin, oat fibre, and RS - IV as soluble and insoluble fibres increased IGP, while an extreme addition of these fibres reduced starch digestibility. These results generated a varied crumb structure with an elevated fibre concentration, leading to a stable S - F web - like structure and disproportional lower IGP levels. In this context, an *in vivo* model study was conducted, reporting that fortified gluten-free bread (high DF content), formulated by mixing acorn flour and chickpea flour which significantly lowered GI (97.4) *vs.* control bread (159.2). This indicates that the stable S - F interactions affect IGP (Gkountenoudi-Eskitzi et al., 2023).

According to the literature, one of the mechanisms of DF in inhibiting the enzymatic interaction between starch and digestive enzymes is its viscosity (Zhang, Sun, & Ai, 2022). Soluble DFs such as gums, pectin, and psyllium impact starch digestion and glucose absorption, resulting in a low IGP for DFs. Initially, the viscosity of soluble DFs is due to physical entanglements, which impede the movement of surrounding solvents. The molecular weight, chemical structure, hydration characteristics, and solubility of DFs all play an important role in determining their high viscosity. It was also assumed that digesta produced during starch hydrolysis increases viscosity, limiting enzyme permeability to starch substrates and mixing efficiency. Furthermore, interactions between DFs can vary the transit duration of chyme in the upper intestine, reducing gastric emptying rate and altering small intestinal transit. Few DFs also have water-holding and swelling capabilities, which prevent physical binding, delay stomach emptying, and promote satiety. Overall, the delayed stomach emptying rate with enhanced viscosity contributes significantly to reduced GR. Furthermore, several reports on DF have highlighted the concept of physical entrapment within starch granules, which indicates reduced digestibility. For example, He et al. (2020) demonstrated that the incorporation of guar gum induced the molecular structuring of micro-extruded rice starch granules, resulting in single-helical and double-helical structures and altered crystallinity. Another study noted a low concentration (0.03 to 0.15%) of guar gum in lotus seed starch maintained a higher crystalline short-range order structure while a higher concentration (0.30 to 0.90%) led to reduced crystallinity and lower IGP (Zheng et al., 2019). Recognizing the impact of DF in regulating hyperglycemia has become a novel holistic approach that requires a deeper understanding of the dynamics and relationships between starch and DF within a FM.

3.1.4. S - Ph interactions

The role of polyphenols in regulating IGP is crucial due to their significant presence in the FM of various plant species as secondary metabolites. They are mainly categorized into two groups: flavonoids and non-flavonoids. Flavonoids include those with C6-C3-C6 structure, while non-flavonoids comprise phenolic acids (PhA), such as hydroxybenzoic and hydroxycinnamic acids. In natural food sources, the bioactivity of phenolic compounds affects starch digestibility through their physical interactions, ultimately forming barriers against enzyme diffusion. At a molecular level, however, different types of hydrogen bonds, electrostatic interactions, and hydrophobic effects determine S - Ph interactions, resulting in inclusion and non-inclusion complexes. A previous study by Li, Pernell and Ferruzzi (2018) explained diverse functional and structural aspects of S - Ph interactions. It showed that phenolics binding to starch molecules enable stacking interaction between aromatic residues of polyphenols and starch pyranose ring *via* 2–3 weak CH-π bonds, forming stable hydrophobic interactions. Further, complexation with proanthocyanidins (PA) modulates starch molecule crystallinity and reorganizes skeletal α-1, 4 glycosidic linkages. Therefore, the V-type inclusion complex formed between starch and phenolic compounds possessing a stable structure impedes the permeability of digestive enzymes (Chi et al., 2017). Rocchetti et al. (2018) highlighted the modulation strategy of polyphenols in different varieties of pigmented maize under cooking conditions. Using a model system, they compared the results of IGP with yellow maize. A comprehensive analysis of 300 phenolic compounds, with a high content of anthocyanin and phenolic (free and bound form) from maize explained that there is a direct correlation of polyphenols with higher SDS and RS and lower hydrolyzation index (HI) as well as IGP.

Further, a study by Krishnan et al. (2021) reported the complementation of nutraceutical starch and PA in limiting IGP and anti-glycation ability. Another study on black tea leaves by Satoh, Igarashi, Yamada, Takahashi and Watanabe (2015) demonstrated the *in vitro* digestion process by inhibiting the activity of α-amylase, α-glucosidase enzymes, and degradation of disaccharides into monosaccharides in the small intestine *via* hydrophobic interactions among S - Ph (catechins, theaflavins, caffeine) molecules. This ultimately leads to lower post-prandial glycemia. Furthermore, reports by Liu et al. (2017) also described S - Ph interplay using a model study in red rice. The results obtained from this study explained that red rice polyphenols mainly inhibit pancreatic α-amylase activity through hydrogen bonding to the active catalytic sites of pancreatic α-amylase (ASP197, GLU233, and ASP300) and ultimately modify the microenvironments of TRP58 and TRP59, which are directly linked to the lowered bioavailability as well as IGP. Polyphenol-enriched extract from pearl millet (*Pennisetum glaucum*) has reported to inhibit key enzymes involved in post prandial hyper glycemia (α-amylase, α-glucosidase) and regulates hepatic glucose uptake (Krishnan et al., 2022). Docking revealed that 3, 4-Di-OMe luteolin and acacetin as the major flavonoids while salicylic acid, melilotic acid as the key phenolic acids in pearl millet which endorse the anti-diabetic effect. In conclusion, whole grains generate binary interaction among S - Ph molecules in the FM, which limits IGP and enhances the nutritional quality of starch-based foods through a potent hydrophobic inclusion complex.

3.2. Ternary interactions

Recent research on the enigmatic *in-planta* organizational structure of biological macromolecules highlighted that glucose bioavailability, as well as IGP in the human body, is affected by the formation of a ternary complex (Lin, Yang, Chi and Ma, 2020). It is suggested that this supramolecular ternary stable structure is modulated by extrinsic factors such as changes in temperature, pressure, or ionic concentration (Wang, Chao, Cai, Niu, Copeland & Wang, 2020). These characteristics make it easier to regulate the release and improve the bioavailability of FM components, bioactive components, and drug efficacy. In this section, we comprehensively discuss the interactions among the three - macromolecules in a three - polymer blend system in the context of IGP.

3.2.1. Starch – Lipid - protein interactions

Among the three-component interactions of the FM, the dynamics interplay of starch – lipid - protein (S – L - P) has extensively been studied in food nutritional chemistry. The formation of a ternary complex was first proposed by Zhang and Hamaker (2003) based on observing a viscosity peak during a setback in a rapid visco analyzer (RVA). Subsequently, the mixtures of sorghum starch, whey proteins, and fatty acids further confirmed the existence of this complex using size exclusion chromatography, which demonstrated that the set three components (starch, lipid, and protein) can self-assemble.

Two categories of naturally occurring proteins, surface proteins, and granule-associated proteins, play a robust role in interactions with starch. According to the theory proposed by Greenblatt, Bettge and Morris (1995), polar lipids can act as "bridges" between proteins and the surface of starch granules, mediating interactions between proteins and starch. Removing nonpolar lipids through solvent extraction with hexane enhances the polar lipid fraction, strengthening the link between starch and protein through the bridge association (Siaw, Wang, McClung and Mauromoustakos, 2021). Monoglycerides, due to their excellent solubility and emulsifying properties, form complexes with amylose more rapidly than fatty acids, diglycerides, and triglycerides (Chao, Yu, Wang, Copeland, & Wang, 2018). A study by Cornell et al. (2019) suggested that basic amino acids bind strongly and stably with the polar head surface of fatty acids in vesicles. This implies that electrostatic attraction between carboxyl groups of free fatty acids and polypeptides is the basis for the self-assembly of the thermally stable ternary complex, which can modulate IGP in the physiological system. Zhang and Hamaker (2005) proposed three different structural elements that could be involved in the development of ternary complex: 1) starch – free fatty acids (FFA) complex, 2) protein - FFA complex, and 3) disulfide bond-linked protein aggregates. Proteins form a solid barrier that encloses and traps the starch granules, forming a continuous matrix that blocks the accessibility of the enzymes responsible for starch hydrolysis (Kang et al., 2021). Additionally, lipids prevent enzymatic hydrolysis of starch by forming a digestion-resistant structure with amylose (Kang et al., 2021). As a result, these findings collectively lead to reduced starch digestibility and IGP (Krishnan et al., 2020). Several studies in this context have reported various methods for preparing a ternary complex of S – L - P to understand the underlying mechanism. For example, the classical method involves heating in boiling water and overnight cooling at room temperature. However, a thermos - mechanical method using an RVA instrument has proven the most successful (Zheng et al., 2018). It is proposed that a ternary complex with a crystalline structure and V - type amylose helices is situated perpendicularly to the structural axis of lamellar stacks. During hydrothermal, amylose leaches out from the starch granules, forming a closed complex in a laevorotatory fashion with lipids. Then, during the cooling process, degraded proteins interlink with crystalline helices to shape the structure of ternary stacks. Alterations in the torsion angles of the glycosidic linkages occur when amylose conformation changes into a helix, potentially impacting the binding ability of the amylolytic enzymes to amylose (Wang et al., 2020). Studies assessing the structural order in these ternary complexes have shown that they exhibit a higher degree of short-range structural order and long-range molecular arrangement, which follows a typical V-type pattern (Chao, Yu, Wang, Copeland & Wang, 2018). These ternary complexes also have higher crystallinity due to their stacking interaction, which is more stable than binary complexes (Cai et al., 2021). Regarding thermodynamic changes, Zhang and Hamaker demonstrated that the presence of protein in the system leads to a decrease in endothermic enthalpy, indicating that protein components bind to the starch-fatty acid complex and reduce the number of sites available for fatty acids (Zhang & Hamaker, 2004). They also further elaborated on the concept of competitive interactions for the S - L complex in the presence of whey protein. Furthermore, pasting properties of the complexes were investigated to understand the effect of starch type on the formation of starch - lauric acid - βLG complexes. RVA analysis of mixtures revealed that maize - lauric acid and wheat - lauric acid complexes demonstrated a viscosity peak during the setback stages of the RVA pasting profile. The potato starch - lauric acid showed only a rise in the final viscosity, while the waxy maize starch - lauric acid system indicated no such changes (Cai et al., 2021). The intricate interactions among these three elements during food processing play a crucial role in shaping the qualitative attributes of food items, such as flavour, texture, mouth feel, and digestibility (Cai et al., 2021). Similar studies have also investigated the mechanisms of interactions between inherent carbohydrates, protein residues, and lipid molecules in foxtail millet and their impact on *in vitro* amylolysis (Jin, Bai, Chen and Bai, 2019). Observations indicate that the matrix composed of proteins and lipids does not act as a steric barrier but inhibits starch amylolysis in foxtail millet flour.

In summary, understanding the interactions within this ternary stable structure has a significant impact in various applications such as supramolecular nano-carriers (where bioactive supplements and food-sensitive compounds are accommodated in the ternary structure) for medical delivery and formulation of functional food products at an industry level. Therefore, this area deserves more attention. Furthermore, when investigating the complexities of ternary complexes, it becomes evident that interactions between other matrix components, such as starch-lipid-phenol, starch-protein-phenol, or starch-protein-fibre, could be explored in the future to gain a better understanding of how these matrix components interact within the human body. Currently, no such research exists that emphasizes the significance or function of these complexes in terms of IGP. Thus, addressing this gap in research on other ternary interactions could lead to new avenues for application. Future research in the field of FM should aim to establish a basic structure - function relationship that can opens up many new possibilities for its application in the formulation of diverse functional foods for individual needs.

3.3. Quaternary interactions

Quaternary interactions have limited attention in the context of regulating post - prandial glycemia. There is scare information available in the literature regarding the formation of starch-lipid-protein-phenolics (S – L – P - Ph) involving quaternary interactions. Detailed studies have focussed on characterizing the complex structures formed due to proteins, lipids, and phenolics, leading to alterations in the GR of starch components such as amylopectin and amylose. These interactions can occur through various mechanisms, such as the inhibition of carbolytic enzymes, modification of the overall solubility, or enrichment of the RS fraction, ultimately reducing the IGP (Krishnan et al., 2020).

Polyphenols are considered beneficial phytochemicals with numerous pro-health properties. However, phenolic compounds have been found to interact with matrix components like carbohydrates (*e.g.*, starch and DF), lipids, and proteins, resulting in significant nutritional and nutraceutical changes in fortified products (Sęczyk, Świeca, Gawlik-Dziki, Luty, & Czyż, 2016). It has been observed that phenolic compound primarily binds with macromolecules through weak non-covalent interactions, including hydrophobic, H-bonding, and van der Waals bonds. However, irreversible interactions strengthened by covalent bonding are also possible in the case of proteins. Sęczyk, Gawlik-Dziki & Świeca, (2021) conducted a study to investigate the impact of phenolic - FM interactions on the *in vitro* bio-accessibility and total antioxidant activity of a few selected phenolic compounds like catechin, chlorogenic acid, apigenin, gallic acid, quercetin, and ferulic acid. They also examined the effect of these compounds on protein and starch digestibility in fortified white bean paste. They demonstrated that adding phenolics had minimal effect on total DF or total starch. However, phenolic compounds reduced the digestible starch content, leading to a significant decrease in starch digestibility. While few reports have mentioned the possibility of quaternary structure formation in the case of pigmented rice due to the high amounts of polyphenols in the matrix (Wang, Zheng, & Chao, 2019), this area of research remains relatively unexplored.

Krishnan et al. suggested that the formation of a quaternary structure resulting from the composition of the matrix in pigmented rice, combined with starch, could be a plausible explanation for the reduced availability of starch and its impact on IGP (Krishnan et al., 2020). Digestion kinetics demonstrated that matrix components, such as phenolics, play a crucial role in determining the rate of autohydrolysis, depending on their types, *i.e.,* PA in red rice were reported to be far more abundant than anthocyanins in black rice (Krishnan et al., 2020). Both monomeric and polymeric polyphenols exhibited a varying degree of inhibition on starch digestibility, with the extent of favourable interaction closely related to their bio-accessibility and bioavailability.

Polyphenols from five pigmented sorghum flours were tested *in vitro* for their ability to modulate starch digestibility. In the context of protein-phenolic interactions, Świeca, Sęczyk, Gawlik-Dziki and Dziki (2014) unraveled diverse biological and functional characteristics of bread fortified with quinoa leaves. The addition of quinoa leaves influenced digestibility and nutrient content. The digestibility of starch in bread was inversely related to the percentage level of quinoa leaves, while variation in the digestibility of protein was not as significant. Results confirmed that the phenolic compounds and FM interactions significantly impact the quality of fortified bread.

The nutritional profile of a pasta-like product (spaghetti-type) formulated with corn (*Zea mays*) flour supplemented with 30% broad bean (*Vicia faba*) flour (C/BB) and 20% quinoa (*Chenopodium quinoa*) flour (C/Q) was studied by Giménez, Drago, Bassett, Lobo and Samman (2016). The proximate composition and iron, zinc, and DF content were determined. The protein content of the spaghetti-type pasta (C/BB) increased by 100% when corn flour was replaced with broad bean flour in a ratio of 70:30 (*w*/w). When broad bean and quinoa flours were added, the lipid content was increased by >100% compared to the control samples. On the addition of 30% broad bean or 20% quinoa flour, the unsaturated portion was increased by 22% and 42.6%, respectively. DF content was also increased by adding quinoa and broad bean flour. The broad bean and quinoa flour addition decreased true digestibility (TD) values ($P < 0.05$) compared to the control. TD reduced by up to 11% and 14% in C/BB and C/Q spaghetti, respectively. The decreased digestibility could be attributed to factors such as increased tightness of both broad bean and quinoa protein structures, other constituents like minerals and fibre, formation of S - P complexes, cross - linking between proteins, and the abundance of anti-nutritional constituents like saponins, phytates, and tannins.

Desai, Brennan, Guo, Zeng and Brennan (2019) focused on using salmon protein and lipids to alter the GI and protein digestibility of pasta. Salmon fish (*Oncorhynchus Tschawytscha*) powder (SFP) was added to pasta flour in amounts ranging from 5% to 20% (*w*/w). The addition of SFP reduced starch hydrolysis and GI values of pasta significantly. Phenolic compounds were released from pasta during gastric (179%) and pancreatic digestion (133%) with SFP addition. Interestingly, while protein quantity increased with SFP addition, protein digestibility reduced from 86.41% (Control pasta) to 81.95% (20% SFP pasta). The sources of DF, including oat (OB), flax (FB), and apple (AB), were evaluated by Kurek, Wyrwisz, Karp and Wierzbicka (2018), as well as wheat bread fortifiers. Adding oat and flax fibres significantly altered the fatty acid levels. Oleic acid (33.83%) and linoleic acid (24.31%) were the most abundant in oat fibre. Only flax fibre contained a significant amount of γ-linolenic fatty acid (18.32%). Bio-accessibility trials confirmed that the DF reduces the consumption of SFAs. Polyunsaturated fatty acids (PUFA) was the least bio-accessible fatty acid group in the range of 72% in oat fibre to 87% in flax fibre. Regarding GI, the control bread had the highest value (80.5), significantly higher than the oat, flax, and apple fibre values. The addition of all the fibres resulted in a low GI. Apple fibre had the maximum total phenolic value (897.2 mg / kg), while flax fibre had the lowest (541.2 mg / kg). The study fills a research gap by considering the GI, profiling FA, and phenolic acid quantity concerning DF application in bread. However, the quaternary interactions, especially among different matrix components, are crucial elements that impact fortified foods' nutraceutical and nutritional ability. These interactions should be considered during the design and evaluation of fortified products. Therefore, because of the complexity of FM interactions, quaternary interactions remain a deep-rooted concern in food science and technology.

4. Food additives and their role in altering glycemia

As ultra-processed foods have a high IGP and post-prandial GR, FM has been modified with various additives like gelling agents, thickeners, foam stabilizers, aromatic flavouring agents, starch-derived additives and sugar inhibitors (Williams & Phillips, 2021). Few recent studies also highlighted that baryard millet starch, guar gum, whole chia flour (WSF) could be served as a low glycemic gelling or thickening agents to enhance the formation (Bangar et al. 2024, Senna, Soares, Egea, & Fernandes, 2024, Kane et al., 2024). Among which, starch-derived additives have found to significantly affect the IGP. It consists of RS, polydextrose (PD), cyclodextrins (CD), maltodextrins (RMD), and isomaltooligosaccharides (IMO). Of them, RS-IV and PD are mostly produced chemically, whereas IMO, RMD, and CD are produced enzymatically. IMO and CDs are starch related oligosaccharides (SROS) are enzymatically transglycosylated to introduce unconventional indigestible bonds, making them novel starch ingredients with low IGP. On the other hand, PD and RMDs are starch-derived fibres (SDFs), soluble with varying viscosity ranges, promoting various food and beverage applications. Low GI foods adopt RMDs in their formulation due to higher percentage of α- and β-(Akhlaghi, 2022; Althnaibat, Bruce, & Gänzle, 2023), (Akhlaghi, 2022; Althnaibat et al., 2023; Bulut et al., 2023), and (Akhlaghi, 2022; Althnaibat et al., 2023; Bulut et al., 2023; Cai et al., 2021; Champ, 2004; Chao et al., 2018) glycosidic bonds than the native starch, making it resistant to enzymatic hydrolysis, (Adam-Perrot et al., 2009). SDFs are heavily used in functional food industry mainly as a low calorific (sugar or fat) replacer (Himat et al., 2021).

Another major additive that contributes to reducing the IGP of our dishes is acetic acid (vinegar). which has proven to reduce the speed of gastric emptying as well as inhibiting the carbolytic enzymes. When 18 mmol acetic acid, or 20 g of vinegar, was coupled with high GI meals such as white bread, a 35% reduction in GI was seen (Shishehbor, Mansoori and Shirani, 2017). Another recent study has found that when the vinegar was given as a vinaigrette with water and olive oil (8 g), the GI decreased by 11%, which may assist to explain the greater decrease in the GR (Shishehbor et al., 2017). Other than the FM interaction effects, acetic acid has also shown to improve GR by increasing glucose uptake and by mediating transcription factors (Santos, de Moraes, da Silva, Prestes, & Schoenfeld, 2019). Similar effects have also been observed with lactic acid. Considering this its logical to conclude that functional foods involving fermentation which incorporates organic acids have role in lowering IGP and GR (Liatis et al., 2010).

5. Starch quality matrix (SQM): A prediction tool for functional foods

Despite having a comparable energy source, different dietary starch sources such as simple sugars, sucrose, and fructose have very distinct metabolic consequences in our bodies. When consumed in excess, they can lead to various chronic diseases. Recent advancements in this context indicate that it is not just about the amount but also related to dietary forms or starch processing conditions that ultimately govern energy efficiency and *in vivo* glucose bioavailability (Fig. 3). To connect these aspects, we can emphasize starch quality and its matrix interactions, which are crucial for recognizing disease - associated factors and aiding in their prevention. In addition, time - dependent digestive fractions (RDS, SDS, RS) of dietary starches suggest a direct connection to starch quality and GR. Considering this relevance, various quality matrices have been designed in recent years, primarily highlighting high RS / DF intake and low sugar with reduced refined starches. In summary,

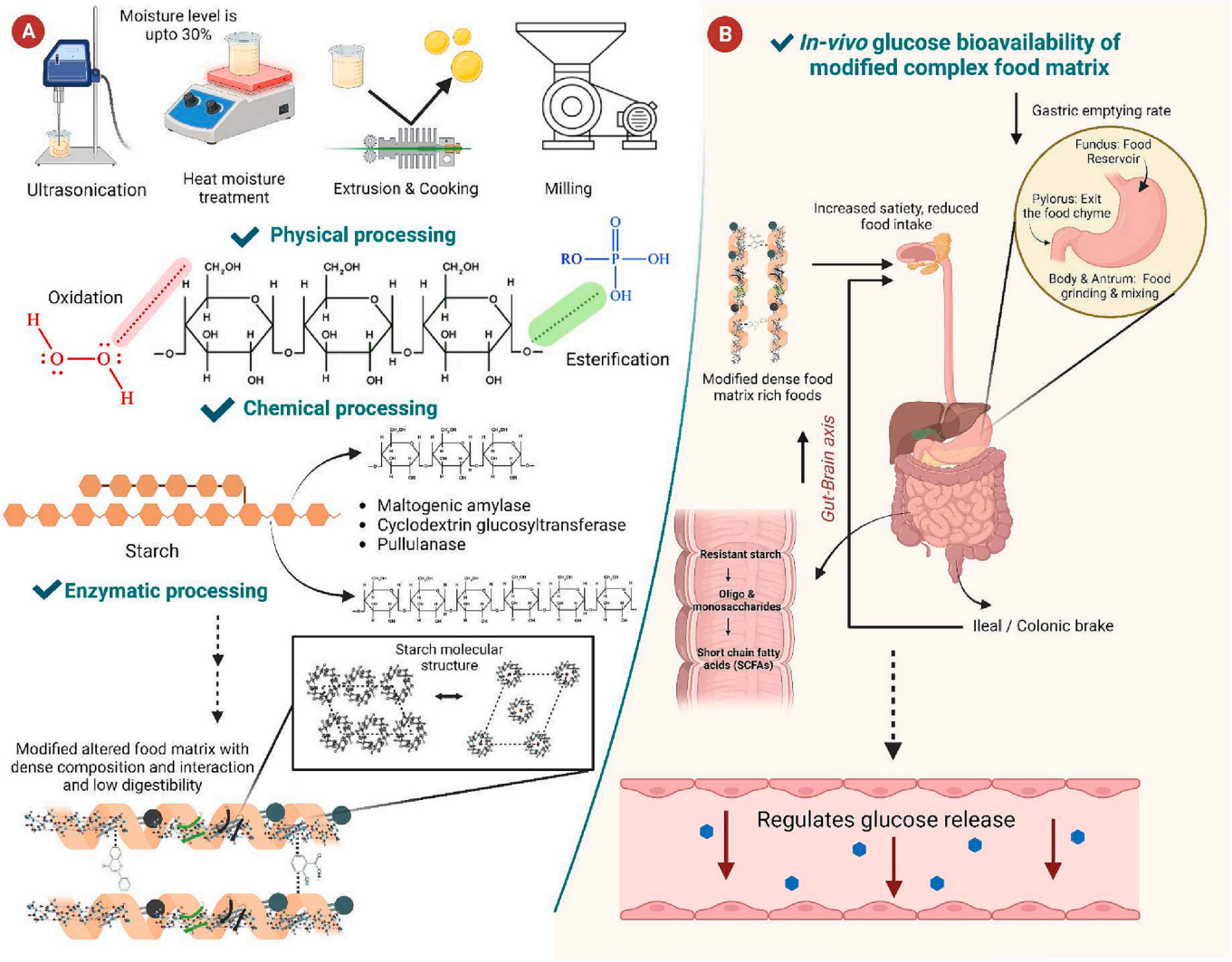

Fig. 3. Different processing techniques alter food matrix components/ interactions and regulate glucose bioavailability. A. Wide range of industrial-scale processing technologies (physical, chemical, enzymatic) has a profound impact on modifying the food matrix with more dense matrix composition, interactions and reduced digestibility. B. The *in-vivo* glucose bioavailability of such modified altered food matrix rich foods reduces the gastric emptying rate, and induces 'ileal/ colonic brake' events which further increases satiety with reduced food intake through enhancing the gut-brain axis. [Created with BioRender.com].

introducing a quality matrix (a measure of starch quality) is critical. According to the American Heart Association, starch quality is defined as the ratio of starch to fibre intake as 10: 1, while World Health Organization (WHO) recommends the consumption of 10 g of starch with 1 g of fibre and <1 g of free sugars (Lloyd-Jones et al., 2010). Several population studies have already revealed robust evidence supporting the correlation between this type of quality matrix and health outcomes, particularly in DFs and free sugars (https://www.who.int/publications/i/item/9789241549028).

However, another group of researchers not only focus on DF consumption but also highlighted the importance of all explanatory variables contributing to the development of high-quality (high SDS, RS) starch sources through conventional breeding. While numerous studies have been conducted to investigate the inter-relationship of those parameters, a rapid predictive tool to examine starch quality at the breeder level is still in its infancy. Consequently, a SQM is highly appealing for assessing all possible parameters influencing starch quality. Especially in whole grains, various contributing factors in FM play a significant role in reducing RDS and enhancing RS content. Among the plant-based contributors, microstructure, botanical origin, molecular configuration, rheological properties, textural classes, physicochemical attributes, gelatinization properties, and various processing techniques are crucial—granule size, shape, bran layer, and channels all impact enzyme diffusion during the digestion process.

Apart from microstructural factors, interactions among FM components have an essential role, which is thoroughly covered in the preceding sections. Additionally, rheological properties affecting starch quality are critical for breeders and the food industry. In RVA, interpreting the viscosity profile is very useful for screening high-quality starch sources. For example, final viscosity, which determines pasting qualities based on cooking and cooling, helps consumers understand food quality. The setback viscosity is defined as the difference between final viscosity and trough and ultimately correlates with the textural properties of starch. Furthermore, the physicochemical properties related to eating and cooking qualities, such as alkali spreading value (ASV), gelatinization temperature (GT), gel consistency (GC), and amylose concentration (AC), vary among different starch sources. Studies have shown increased GC correlates with higher amylose content and reduced glucose bioavailability (Lin et al., 2022). Similarly, lower GT also promotes faster hydration and reduced RS content. Therefore, a consistent correlation between starch quality parameters and glycemic profile is urgently needed to understand how each parameter significantly contributes to improving the quality (high SDS and RS) in starch sources. In conclusion, based on observations, we can

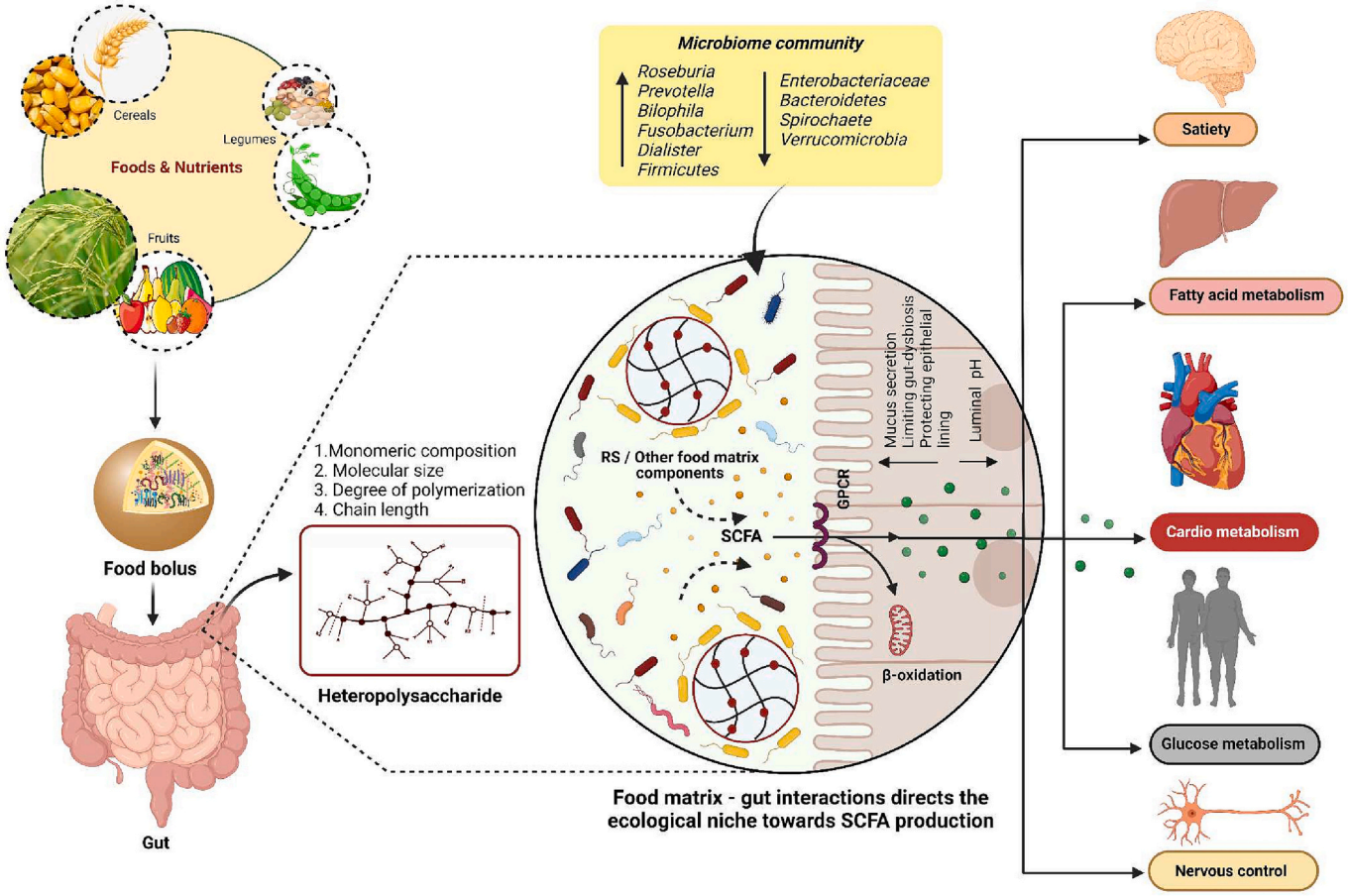

Fig. 4. Role of food matrix components/ interactions in regulating gut-health. Food matrix of functional foods plays a profound role in regulating gut-microbial diversity. The indigestible but fibre - rich matrix components induce food matrix-gut interactions based on monomeric composition, molecular size, degree of polymerization and chain length of heteropolysaccharides which directs the ecological niche of large intestine and produces array of compounds, called short-chain fatty acids (SCFAs). This microbial production of SCFAs, thus, stimulate thick intestinal mucus layer, protects epithelial lining, prevents gut dysbiosis and reduces luminal pH with several barrier function. Additionally, SCFA can bind to the G-protein couple receptors (GPCR) and secrets peptides, and gut hormones that contribute to energy expenditure, glucose metabolism, nervous system regulation in the body and also maintains anaerobic environment in the gut-lumen. Few gut-microbial communities have been represented in the figure with upward arrow (), showing abundance during food matrix interactions and downward arrow (), showing decrease during matrix interactions) to give an overview of selected gut-microbiome features in starch-based food matrix interactions. [Created with BioRender.com].

assert that for the screening and analysis of extensive starch sources, a cost-effective statistical model like SQM is of utmost importance for breeders and consumers in the food industry.

6. FM interactions and gut microflora: An added dimension of glucose metabolism

Gut flora is crucial in preventing and controlling glucose metabolism through multiple pathways targeting the intestine, liver, and pancreas. Despite the complexity of this relationship, there is a growing interest in how FM interacts and coordinates with microbiota in managing glucose imbalance. A series of seminal studies have highlighted that among the various factors that alter the gut microbiota composition, FM plays the primary role (Rodriguez, Benninghoff, Aardema, Phatak and Hintze, 2019). Substantial evidence supports the role of specially designed FM with finely tuned interactions in altering gut composition and metabolite landscape (**Fig. 4**). Incorporating components like RS, which has been proven to alter the IGP, has been found to alter the gut-microbial community and fermentation density. Binary interactions like S - L complexation have been observed to impact *in vitro* fecal fermentation outcomes, with the major effect dependent on starch assembles rather than lipid type (Zhou et al., 2021). S - L complexes significantly enhance the abundance of beneficial gut microbiomes like *Prevotella* and *Roseburia.* On the other hand, S - L complexes, with myristic acid and lauric, stimulate the growth of some harmful gut microbiomes like *Dialister*, *Fusobacterium,* and *Bilophila*. In contrast, other fatty acids like stearic and palmitic acids did not exhibit such effects (Zhou et al., 2021).

S - P binary interactions have also been found to modulate and impact gut flora and lipid metabolism. Binary interactions, as reported by Wang et al. (2022), found a greater presence of *Lachnospiraceae* influenced by the starch and protein interactions. Lv et al. (2016) reported the potential for inhibiting pathogenic bacteria like *Enterobacteriaceae* by altering the S - P interaction through a 3% reduction in protein content. Lower protein concentration in the starch-rich matrix was associated with a higher frequency of *Firmicutes*, while *Verrucomicrobia, Bacteroidetes*, and *Spirochaetae* decreased (Chen et al., 2019).

While the impact of different fibres on gut flora has been thoroughly investigated, S - F interaction studies are still limited. Fibres, which are microbiota-accessible carbohydrates based on their discrete structure (monomeric composition, molecular size, linkage, degree of polymerization) and physical characteristics (solubility, insoluble three-dimensional structure) induce a divergent and precise directed effect on gut composition as well as a directed shift in the output (SCFAs) (Bulut, Cantu-Jungles, Zhang, Mutlu, Cakmak, & Hamaker, 2023). Outcomes of fibre fermentation, such as pH lowering, mucous layer protection, limiting gut-dysbiosis, and SCFAs, have demonstrated health

benefits (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016). Mechanistic studies using *in vivo* models have also shown beneficial effects of SCFA like butyrate in maintaining GI barrier integrity, quenching oxygen at the epithelial interface, and exerting immune-modulating effects. This underscores the fact that, beyond the type of fibre, the structure of fibre within the matrix plays a critical role in delivering beneficial effects.

The prebiotic DF upon microbial fermentation within the gut, release SCFAs such as acetate, propionate, and butyrate that have been recorded for their health effects. This fosters a balanced environment for the microbiota and beneficially affects the intestinal health of an individual. Literature has shown that RS, resistant oligosaccharides, fructans, inulin, and galactans serve as a potential source of prebiotics, as their digestion and absorption within the gastrointestinal tract (GIT) is slow, enabling them to stay long and can serve as a substrate for the gut bacteria for fermentation. The chain length, glycosidic bonds, degree of polymerization, and solubility highly influence the digestibility of resistant oligosaccharide and their fermentation capacity making them widely acknowledged prebiotics. Polydextrose and pectins are non-starch polysaccharides metabolized more slowly due to their complexity. Another type of starch is RS which has varied sources and categorization into various types (type I, type II, type III, type IV, and type V) and also exhibits prebiotic properties within the intestine. Apart from this, polysaccharides other than carbohydrates such as chitin and lignin are present in the food. Lignin is both a DF and phenol but research investigating their prebiotic role, especially in humans remains limited (Rezende, Lima, & Naves, 2021).

Recent studies also mentioned that modified matrices like soluble crosslinked arabinoxylans, cross linked type IV RS, modified pectins creates a directed ecological niche for butyrate producers (Zhang et al., 2019). As both matrix components and the microbial species do not function in isolation but form complex networks through mutualistic and competitive interactions, it's vital to include FM-microbiota interaction studies while validating newer food prototypes. Thus, by understanding the complex process by which FM governs gut metabolism and homeostasis, a new dimension may emerge in the use of DF to design functional foods that could manage chronic glycemia-induced abnormalities and gut-health-associated diseases as well. The interaction of FM and its effects on gut microflora are still relatively unexplored, and complex interactions like ternary and quaternary have not yet been reported.

7. Existing lacunas of FM interactions

FM interactions are ubiquitous in any whole food system and have a significant role in metabolism, particularly nutrient bioavailability. Previous studies have already gathered ample knowledge regarding FM's chemistry, physiology, and prospective health benefits. However, the interconnections within the matrix have only recently become separate research domains. Consequently, information on how matrix components interact, facilitate nutrient bioavailability, and contribute to human health has been sporadic. The complex chemical interplay of each component within foods and their interaction with the gastrointestinal tract makes it a daunting task to fully understand their functions independently (Zhang et al., 2020).

Nevertheless, current methodologies used to analyze and assess matrix structure are often ineffective and may not always correlate with physiological outcomes in *in vivo* results (Zheng, Wang, Wang, Chen, & Zhou, 2020). The existing gut-nutrient interaction studies are mainly focused on gut microbiota composition and metabolism using murine models considering the merits like similarity in the GIT physiology, well-curated genetic information availability, less inter-individual variation due to inbreeding, and so on. However human studies are more relevant in such cases considering the fact that the relative size of GIT-associated organs and especially villi architecture differs. Many more gut-microbial fermentations of such complex FM occur in the caecum of animals rather than in colon-like humans. The most critical boost for clinical trials is the host-specificity aspect of gut microbiota and it may not be recapitulated totally in any other *in vivo* models (Ward, Benninghoff and Hintze, 2020). Therefore, to address these gaps in knowledge, this review aims to compile existing knowledge on FM and serve as the foundation for future studies. While FM's role in altering gut flora is exciting, a better understanding of how the molecules involved in these interactions can be measured and correlated with their impact on gut microbiota composition is crucial. Besides, it's intriguing to decipher how different FM interactions may be linked to microbiota - produced metabolites that affect glucose regulation (Zhou et al., 2021). Since the integrity of the intestinal structure and the dynamic balance of the microbiome are essential for gut endocrine function and digestive performance, fine-tuning FM interactions in this direction could open up new dimensions in functional foods. Altered matrices with superior fibre components like RS and others have been reported to influence gut landscape. However, results have varied depending on the specific location within the gut, highlighting the need for more in - depth studies that consider location - specific effect.

8. Conclusion and future perspectives

In conclusion, this review compiles information on how matrix components and their intricate relationships influence or restrict IGP. In the recent era, it is worth noting that the impact of FM interactions plays a crucial role in designing and manufacturing functional foods with new attributes for consumers, researchers, and producers. To understand the concept of '*FM*' and its effect on nutrient or glucose bioavailability, nutrition and food scientist have endeavored to plan how a single matrix component in solution may exhibit a more diverse effects than in whole food. Specifically:

1. A single matrix component or a group of matrix components can influence each other's activity through various modes of action, such as additive, synergistic, masking, antagonistic, or neutralizing effects.
2. FM components can affect the functionality and performance of the whole food by influencing the bio-accessibility, bioavailability, and metabolism patterns of nutrients.
3. Each food elements (matrix) such as lipids, proteins, phenolic compounds, minerals, DF, prebiotics, can influence nutrient or glucose release through stable, intricate, tangled binary, ternary, and quaternary interactions. These interactions ultimately inhibit salivary and pancreatic enzyme actions, regulates IGP, and impact the intestinal microbiome environment, contributing to a healthier lifestyle.

However, current research mainly focuses on the effect of individual FM components (lipid/ protein/ phenolics/ fibres) on glucose release, while more information on enzyme inhibition and IGP in the presence of multiple FM components is crucial. Therefore, this approach to studying FM could be of significant value in assessing food performance.

In this context, foods with low IGP offer profound health benefits such as improved gut microbiota, enhanced micronutrient bioavailability, and better glucose and lipid metabolism. Binary interactions between starch and lipid molecules play a significant role in applications such as bread making, prevention of various non-communicable diseases like colon cancer, type 2 diabetes, chronic hyperglycemia, respiratory disease, cardiovascular disease, fat replacement, and edible biodegradable film production. Additionally, these binary, ternary, and quaternary complexes notably influence starchy foods' dietary quality and functional properties, developing novel food products with distinct functionalities, such as plasticizers and thickeners. Notably, these stable complexes have potential applications as fat replacers in low - calorie or keto diets. Further, the formation of RS in the FM has also been recognized as nutritionally improved functional food component.

Furthermore, significant research efforts should be made to evaluate each matrix interaction and its mode of action. However, processing techniques, intrinsic biological factors, FM characteristics must be considered to alter and predict its function within a whole matrix. While formulating novel and unique FM, the bioavailability of each component, organoleptic characteristics that modify food nutritional properties must be considered. The impact of such functional foods with improved FM on health needs to be evaluated and validated by *in vivo* model studies. Therefore, designing and engineering a new FM that ensures the controlled release of essential matrix components is fundamental to creating "healthy and functional" foods. A new rigorous strategy for characterizing and investigating interconnections among each food component will enhance our understanding of food product functionality, nutrient bio-accessibility, bioavailability during oro – gastro - intestinal assimilation, and development of improved *in vitro / in vivo* models for nutritional evaluation.

Author contributions

Debarati Mondal, Monika Awana, Shreya Mandal and Kangkan Pandit drafted the original manuscript. Archana Singh and Sijo Joseph Thandapilly provided critical inputs and corrections. Veda Krishnan helped with the conception and structural design of the manuscript and finalized the manuscript for submission**.** Cyprian Omondi Syeunda edited the manuscript for better readability in accordance to native English speaker/reader.

CRediT authorship contribution statement

Debarati Mondal: Writing – original draft. **Monika Awana:** Writing – original draft. **Shreya Mandal:** Writing – original draft. **Kangkan Pandit:** Writing – original draft. **Archana Singh:** Writing – review & editing. **Cyprian Omondi Syeunda:** Writing – review & editing. **Sijo Joseph Thandapilly:** Writing – review & editing, Supervision. **Veda Krishnan:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this work.

Data availability

Data will be made available on request.

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Further-reading

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