Interactions among starch, lipids, and proteins in foods: microstructure control for glycemic response modulation

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Abstract

In real food, starch is almost always forming part of a matrix with lipids and proteins. However research on this ternary system and interactions among such food components is scarce so far. The control of food microstructure is crucial to determine the product properties, including sensorial and nutritionals ones. This article reviews the microstructural principles of interactions among starch, lipids, and proteins in foods, as well as their effect on postprandial glycemic response, considering human intrinsic differences on postprandial glycemic responses. Several lines of research support the hypothesis that foods without rapidly digestible starch will not mandatorily generate the lowest postprandial glycemic response, highlighting that the full understanding of food microstructure, which modulates starch digestion, plays a key role on food design since a nutritional view point.

Keywords: starch digestion, food microstructure, glycemic response, genetic background

1. Introduction

Starchy foods are products having a high starch fraction, but it does not mean that other matrix constituents are absent. Examples of starchy foods are bread, pasta, baked products, etc. All these foodstuffs are matrices built mainly by starch, proteins, and lipids, but type of ingredients and processing conditions affect their microstructure, and finally their properties. In this way, current evidence suggests that the knowledge of binary interactions between starch, lipid, and protein are not sufficient to understand the properties of a formed microstructure neither its actual effect on starch digestibility when these three constituents are all together in food. As synergistic or not lineal effects derived from ternary interaction among starch, lipid and protein may be present, extrapolation of observations done in binary systems would be not the most suitable way to explain the physical and nutritional properties of foods (Zhang & Hamaker, 2003, 2004, 2005; Zhang et al., 2003, 2010; Jacobs et al. 2009). However, detailed studies about microstructure of ternary (or more complex systems) are scarce so far. In addition, current evidence suggests that a linear relationship between food microstructure, digestibility, and glycemic response (nutritional effect) is not always clearly found due to genetic or physiological differences among consumers, which finally determine the actual nutritional effects of foods (Englyst & Englyst, 2005; Cummings & Englyst, 1995).

This paper reviews the current knowledge about interaction among starch, lipids, and proteins in starchy foods, as well as its role in starch digestibility and glycemic response. We will also emphasize the importance of genetic background of consumers in determining the postprandial

glycemic response. It is also discussed the idea that a small fraction of very rapidly digestible starch could be useful to modulate the glycemic response.

2. Starch and its digestibility

Starch is a complex carbohydrate produced by plants to store energy generated through photosynthesis. It is constituted by glucose units which form two types of biopolymers: amylose, which is linear (glucose with Ŭ-1,4 bonds), and the amylopectine, which presents branches and a much higher molecular weight (glucose units linked by two types of bond: Ŭ-1,4 in the linear sections, and Ŭ-1,6 in the branching points). Both polymers are joined forming discrete granules of different size, shape and amylose/amylopectine ratio, depending on the botanic origin (Sajilata et al., 2006; Copeland et al., 2009). Starch is especially abundant in legumes, tubercles and cereals; all them highly consumed by human beings worldwide, hence it is considered as their main energy source (Copeland et al., 2009).

When consumed, starch is digested both at mouth level and in the small intestine by the sequential action of Ŭ-amylases (salivary and pancreatic), and the concerted action of enzymes such as the Maltase-Glucoamylase (MGAM) complex, and the Sucrose-Isomaltase (SI) complex (Santos et al., 2012; Nichols et al. 2009). The final process of starch digestion to free glucose is completed in the small intestine brush border, being glucose efficiently absorbed in the small intestine and transported to the blood stream.

Undigested starch passes to the large intestine, where it may be fermented by the microbiota, thus producing short-chain fatty acids, which have been related to beneficial health effects (Dona et al., 2010; Cummings & Englyst, 1995). Starch digested into glucose and having effect on

postprandial glycaemia is so-called ñdigestibleò, whereas the starch passing to the large intestine is known as ñresistantò. Digestible starch, on its turn, is divided into rapidly and slowly digestible. Slowly digestible starch produces more moderate changes in the postprandial glycaemia, whereas rapidly digestible starch produces rapid changes in the glycaemia, even similar to that produced by simple sugars, characterized by a fast increase in blood glucose concentration up to maximal high levels (Englyst et al., 1992). A classification of starch based on its digestibility is shown in Table 1.

Much interest has been generated in the identification of food that, when consumed, leads to a lower glycemic response; that is, a lower change in blood glucose concentration after food ingestion (postprandial period). This interest is based on the growing scientific evidence suggesting that exaggerated postprandial glycemic response would be a risk factor for several chronic no transmissible diseases, such as diabetes and cardio-vascular diseases (FAO/WHO, 1998; Brand-Miller, 2003; Thomas & Wolever, 2003; Jakobsen et al., 2010; Larsen et al. 2010). The postprandial glycemic response (glycemic index, glycemic load) of a great variety of foods is known; their values may be considered as referential (Foster-Powell et al., 2002; Dona et al., 2010). Several *in vitro* methods have been developed to estimate glycemic response; they may significantly differ in their methodology and not always predict *in vivo* glucose homeostasis precisely since such methods do not include the high number of factors that take place in the physiological responses to carbohydrate-rich food (Woolnough et al., 2008; Priebe et al., 2008).

3. Food microstructure and starch digestion

Concerning factors determining starch digestibility and glycemic response, two main groups may be identified: those related with consumer (for example, physiological state, genetic background) and those related with food characteristics (for example, composition, structure) (Cummings & Englyst, 1995).

Among those factors related to food, it has been observed that their microstructural and mechanical properties (affected in turn by microstructure) may act at various levels, affecting starch digestion rate (Turgeon & Rioux, 2011; Parada & Aguilera, 2011a). In the mouth, it has been observed that the size reduction of food, its disintegration and the rate of starch hydrolysis depends on the chewing time as well as the physical characteristics (determined by microstructure) of ingested food (Hoebler et al., 1998). Microstructure may also delay the gastric emptying by presenting structures which are more difficult to be disintegrated inside the stomach, thus affecting the rate of the whole digestion process in the intestine. Furthermore, the matrix where starch is in the intestine and the compound intimately acting with the starch may retard or even avoid its contact with the digestive enzymes, hence reducing its digestibility. Finally, the degree of gelatinization/retrogradation of starch, which is a function of the food processing and storing conditions, affects the total digestible starch proportion, since while the gelatinized starch (amorphous structure) is rapidly digested, those molecules in native crystalline state or retrograded are of low digestion (Fardet et al., 1998; Mora-Escobedo et al., 2010; Parada & Aguilera 2011a).

Although it is known that microstructure affects starch digestibility, there is much to be understood concerning involved mechanisms and changes magnitude. This information is highly

relevant for the development or election of food having specific nutritional properties (Parada & Aguilera, 2011a). In this sense, the appropriate characterization and quantification of interactions among the different food constituents is a very important aspect from a structural, rather than a compositional perspective (Aguilera, 2005; Jacobs et al., 2009).

4. Interactions among starch, lipids, and proteins in food, and their effect on starch digestion

Many starch-rich foodstuffs (pasta, bread, etc.) are basically formed by starch, protein and lipids, whose contents may be relatively simply determined through standardized methods. Nonetheless, many times this information is not sufficient to understand the digestibility of a product (Riccardi et al., 2003; Mourot et al., 1988). In many cases, this difference derives from the microstructure formed during the manufacturing process; however, information about how these three ingredients interact in food and the real effect of the structures present on starch digestibility is still scarce (Zhang and Hamaker, 2003, 2005).

Food constituents interact forming structures showing particular properties, which has been deeply studied in binary systems, such as starch-lipids (Tang & Copeland, 2007); nevertheless, in ternary or more complex systems, information is still rather limited (Zhang & Hamaker, 2003; Zhang et al., 2010). For example, the complex amylose-lipid is known to form two types of structures: I and II. Structure type I melts at a temperature 10 or 30 degrees lower than the melting temperature for structure type II. Likewise, the structure type II may be detected by X rays crystallography; whereas type I is not detectable through this technique, which suggests that this type is probably in an amorphous state. Amylose may form structures both type I and II,

whereas amylopectine will probably bond a lipid only through individual chain. In general many factors affect the formation of the complex starch-lipid.

The type of starch-lipid formed structure depends on the lipid type and the conditions under which it is formed. Lipids with charged head groups have a lower tendency to form type II structures (Eliasson & Wahlgren, 2004). In other cases, both form types, I and II, have been reported, depending on how the complexes are formed. Complexes type I are dominant if formation occurs rapidly and at low temperatures (between 70-80 °C), whereas the most ordered complexes type II are favored if the complex formation happens at high temperatures (Eliasson & Wahlgren, 2004).

Complexes between amylose and lipids (fatty acids, lysophospholipids and monoacylglycerides) may significantly modify starch properties and functionality; for example starch solubility is reduced in water, rheological properties of pasta are altered, swelling capacity is reduced, gelatinization temperature is increased, retrogradation is retarded and susceptibility to enzymatic hydrolysis is reduced (Copeland et al., 2009).

In a study on the effect of superficial components of starch granules (non-carbohydrate surface components), Debet & Gidley (2006) classified starch as of fast swelling (e.g. waxy maize, potato), of slow swelling that may be transformed into fast swelling by extraction of superficial lipids and proteins (e.g. wheat, maize), and of limited swelling not affected by protein/lipid extraction (e.g. high amylose maize/potato). Additionally, Noisuwan et al. (2011) observed that milk proteins, located in the interface or in empty spaces (channels, holes, etc.) of starch granules (rice), may restrict water diffusion inside the granule during its gelatinization.

On the other hand, there are studies about how gluten proteins may form matrices covering starch granules, thus avoiding both their gelatinization and their contact with digestive enzymes; all of which makes digestibility more difficult (Auger et al., 2008; Li et al., 2007; Parada & Aguilera, 2011b). Pasini et al. (2001) observed in bread that at high cooking temperatures (>180 °C) *in vitro* digestion of wheat protein is significantly limited due to the formation of protein aggregates having high molecular weight stabilized by strong irreversible interactions, different from SS and/or hydrophobic bonds that may be present in processes at low temperatures (<100 °C). Additionally, it has been observed that an ñadequateò kneading/mixing maximizes the formation of a protein matrix (gluten) through the action of disulfide bonds. However, if an excessive kneading/mixing is carried out, that matrix loses firmness when the bonds break and glutenin particles are dissociated into smaller fragments, which would contribute to the accessibility of digestive enzymes and would enhance starch digestibility (Parada & Aguilera, 2011b). Hence, the processing (mechanical, thermic, etc.) may affect the interaction level among the protein molecules, affecting digestibility of the whole system.

As previously stated, there is little information about microstructures of ternary systems formed by starch, protein and fatty acids and their effect on starch digestibility. Starch and proteins are two important biopolymers whose interaction is inherently difficult; nevertheless, the presence of free fatty acids (FFAs) has been observed to favor the formation of ternary complexes, where possibly three different structural elements act: formation of complexes starch-FFA, formation of complexes protein-FFA, and the disulfide bond-linked protein aggregate (Zhang et al., 2003, 2005).

Zhang & Hamaker (2003) studied interaction between sorghum starch, serum protein and FFAs (20:2:1, p/p/p) by means of a rapid visco-analyzer (RVA). The authors observed the presence of a noticeable peak in viscosity (during the phase of cooling) when the three components are present in the system, different from binary systems constituted only by starch and protein or FFAs, where the viscosity peak was not observed or was significantly lower. Additionally, it was observed that both the sequence of components addition to the ternary system and the fatty acid used (probably related to the unsaturation degree) affected peaks.

Furthermore, it has been observed, in model systems, that the presence of serum protein diminishes the formation of the complex starch-FFA; nevertheless the crystalline order of this complex (V type crystals) was better defined. These changes were related to the formation of the ternary complex among starch, FFA and protein, since movement of basic structural elements of the complex amylose-FFA would be restricted, thus helping the formation of more ordered complexes and diminishing the opportunity to form new complexes between amylose and FFAs (Zhang & Hamaker, 2004).

In another study, Zhang et al. (2010), concluded that FFAs may act as a bond between amylose and protein molecules, which are thermodynamically incompatible, and whose functional carboxyl group is essential for the formation of a stable ternary complex. The authors suggest that electrostatic interactions among carboxyl groups, possessing negative charge, of the FFAs and the poly-ionic protein are the basis for the self-assembly of the complex.

Concerning the effect of lipids on digestibility and starch glycemic response, it has been proposed that the lower glycemic response observed when lipids are ingested with starchy food

may be related to various factors, such as: delay in gastric emptying, higher insulin response, lower glucose absorption in the small intestine and a lower accessibility to the enzyme alpha-amylose (Latgé et al., 1994; Fardet et al., 1999; Henry, 2009). Fardet et al. (1999) studied the effect of lipids in the accessibility of alpha-amylose in wheat-based food. The authors observed that, depending on the food and the lipid emulsion used, the lipid presence may not affect, diminish or slightly enhance starch digestibility, without presenting high differences in any case. Though, in that experiment, the lipid fraction was added to the food (they were impregnated) after their elaboration; that is, after forming the global structure.

Lipid presence (interaction starch-lipid) may also avoid gelatinization by inhibiting hydration of amylopectin chains, and retard retrogradation, which affects its digestibility (Henry, 2009).

Different processes may generate different and/or deeper changes in physical properties (microstructural) in food, thus affecting starch digestibility and, finally, glycemic response (Lee et al., 2005).

There are many studies about how processing and storing conditions affect starch digestibility and glycemic response. Specifically concerning potato, it has been observed that any process involving heating above gelatinization temperature generates very high glycemic responses, similar to those reached by glucose; while a storing period at low temperatures significantly diminishes that response due to the retrogradation process; some variations may be observed depending on the specific conditions of the experiment (Tahvonen et al., 2006; Foster-Powell et al., 2002; Soh & Brand-Miller, 1999; Garcia-Alonso & Goñi, 2000; Fernandes et al., 2005; Kingman & Englyst, 1994). Nevertheless, there is little information about more complex

systems, involving interactions among different bio-polymers, and about the involved micro-structural changes (e.g. interaction degree of constituents); there is not much clarity, either, about the real effect of these changes on the glycemic response (action level).

5. Iintrinsic factors to be considered for personalized nutrition

In order to a full understanding of postprandial glycemic response, the sole comprehension of food characteristics appears to be insufficient, being crucial the knowledge of consumer profile in regard with its genetic background, metabolic status, etc. In general, variability of the glycemic response among individuals after a carbohydrate-rich meal is known to be larger than within-subjects variability (Frost & Dornhorst, 2002). This variability can be explained by several intrinsic factors such as disease status and genetic profile (Cummings & Englyst, 1995), and even others factors like degree of particle breakdown resulted by mastication can affect significantly, hindering the outcome analysis (Ranawana et al., 2010).

5.1. Type 2 diabetes

As defined by the American Diabetes Association, ñDiabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both" (American Diabetes Association, 2010). In type 2 diabetes, the most common type of diabetes, there is a relative insulin deficiency caused by a combination of pancreatic ß-cell defect coupled to insulin resistance, frequently accompanied by inadequate glucagon suppression after meals, increased hepatic glucose output, and decreased amplification of insulin secretion mediated by gut hormones responsible of the incretin effect, such as GLP-1 or GIP (DeFronzo, 2004).

5.2. Salivary amylase activity and variability of postprandial glycemic response

Although many genetic loci have been previously implicated in type 2 diabetes (Hiveert et al. 2011), there are no clear examples of interaction between food microstructure and genetic susceptibility to type 2 diabetes. In this context, genetic variation of the salivary Ŭ-amylase, the first enzyme that initiates starch digestion in the oral cavity, emerges as a possible genetic marker related to glycemic response to food microstructure. The salivary Ŭ-amylase is the. It is sometimes stated that the action of salivary Ŭ-amylase on starch digestion is relatively unimportant compared to the effect of the pancreatic Ŭ-amylase or other enzymes involved in carbohydrate digestion in the small intestine given its short action that is inhibited by the low pH in the stomach (Frayn, 2010). The salivary Ŭ-amylase is encoded by the *AMY* gene that shows a particular type of genetic polymorphism called Copy-Number Variation (CNV), which means that the number of functional gene copies may range from 2 diploid copies of the *AMY1* genes (1 of paternal and 1 of maternal origin) to as many as 16 copies (Perry et al. 2007). Interestingly, it has been found a positive correlation between *AMY1* CNV with Ŭ-amylase concentration or activity in saliva. Additionally, a wide range of CNV of the *AMY1* gene has been found in the different populations (Perry et al. 2007; Santos et al. 2012)On the other hand, a positive selection of *AMY1* copy number during evolutionhas been proposed, given that *AMY1* copy-number is higher in current populations evolved under high-starch diets versus *AMY1* copies in populations evolved under low-starch diets (Perry et al. 2007; Santos et al., 2012).

It has been recently reported that high endogenous salivary Ŭ-amylase activity due to high *AMY1* copies is associated with improved glycemic homeostasis (lower glycemic response is achieved)

following starch ingestion in healthy, normal-weight adults (Mandel & Breslin, 2012). This observation is in contrast with the previous idea that activity of salivary Ŭ-amylase during chewing (mouth phase) plays a minor role in glycemic impact of foods (Woolnough et al., 2010). According to Mandel & Breslin (2012), individuals having low activity of salivary Ŭ-amylase (an low CNV of *AMY1* gene) may be at greater risk for glucose intolerance after ingesting starch-rich diets compared to subjects with high levels of endogenous salivary Ŭ-amylase. The authors suggest that the early release of starch digestion products in the oral cavity may signal the body to prepare for incoming starch and the ensuing glucose through the vagal activation, resulting in an early insulin release that can be termed preabsorptive insulin release (PIR, also known as cephalic phase insulin release). This type of preabsorptive secretion has been clearly characterized in the cephalic release of other hormones such as the pancreatic polypeptide (Teff, 2010). It has been described that, although PIR is weak and probably represents only a minor proportion of secreted insulin, it may have an important influence in the glycemic response (Ahrén & Holst, 2001). Butterworth et al. (2011) suggest that sweet receptors in the upper sections of the gastrointestinal tract could detect any maltose formed from starch by salivary amylase and if coupled to the release of a number of signaling peptides such as GLP-1 and PYY, influence CNS-mediated control of gastric emptying, insulin secretion and appetite. Additionally, it is stated , that in the case of maltose created in the mouth, there could also be a feed-forward effect in such a way that after its detection by receptors in the gastrointestinal tract, the signals could be interpreted as a warning that a starch containing meal has been consumed and so lead to increased secretion of pancreatic amylase to digest the expected starch load.Moreover, it has been observed that starch hydrolysis in the mouth also depends on the

initial structure of the food as well as in the breakdown of solid food (Hoebler et al., 1998), suggesting that the food microstructure could affect the PIR, as well.

On the other hand, it has been observed that the consumption of slowly digestible starch does not always result in a low glycemic response since if glucose enters the circulation at a slower rate, less insulin is secreted to keep glucose concentrations beneath an acceptable limit, so resulting in a slower uptake of glucose into tissues, and then in a high glycemic response (Eelderink et al., 2012a,b). This fact, together with the possible effect of PIR described above, supports the idea that foods having a small fraction of very rapidly digestible starch could help to achieve a lower glycemic response than one without this fraction, due to the early secretion of insulin.

5.3. Proteins and satiety

Additionally to the structural effect of proteins (that finally affects the stomach emptying rate and starch digestion), they can also help to regulate posprandial glycaemia through the regulation of food intake and satiety. Several mechanisms have been proposed to explain the satiety effect of proteins: increases in the concentrations of hormones involved in satiety signals, increases in energy expenditure, variations in amino acid concentrations, and increases in the process of gluconeogenesis; and it has been shown that high-protein foods cause higher sensory-specific satiety and decrease of the feeling of hunger than similar low-protein foods (Turgeon & Rioux, 2011; Lang et al., 1999). Hence, this not structural effect of proteins should also be considered when nutritional properties of food related with the control of glycaemia are researched.

6. Conclusions

Although basic interactions of food matrices constituents are known and have been studied, information regarding microstructure of ternary or more complex systems as well as their effect on starch digestibility is still limited. Additionally, there is evidence suggesting that the presence of fatty acids (and their type), besides processing conditions, may affect the physical properties of protein and starch based systems. On the other hand, intrinsic differences among individuals such as copy-number variation of *AMY1* gene might be considered *in vivo* to avoid factors confusion and to determine the actual effect of food microstructure on the glycemic response of starch-rich foods when consumed by different individuals. Figure 1 shows the possible relationship among *AMY 1* gene, food digestibility, and glycemic response, and suggests that a lower glycemic response can be achieved if subject has high copy-numbers of the gene, or the food is designed to have a small (but significant) fraction of very rapidly digestible starch (but ideally avoiding to have a big fraction of rapidly digestible starch) capable to release rapidly a little amount of starch digestion products, resulting in an early insulin release. This full control of food digestion could be achieved by studying interactions among ingredients. All previously stated confirms the necessity to research the food microstructure in ternary or more complex food systems, in order to control starch digestion as well as its real effect on postprandial glycemic response. Finally, more *in vivo* research is required to confirm or refute the system proposed in Figure 1, and to know details about the mechanisms involved in the early insulin release during cephalic phase caused by salivary amylase activity.

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Table 1. Classification of starch based on its digestibility *in vitro*, proposed by Englyst et al. (1992)

Starch fraction	**Definition**
Resistant starch (RS)	Starch not hydrolyzed within 120 min
Slowly digestible starch (SDS)	Starch digested during the period between 20 and 120 min.
Rapidly digestible starch (RDS)	Starch hydrolyzed within 20 min
Very rapidly digestible starch (VRDS)*	Starch hydrolyzed within 1 min

*Fourth category described by Chung et al. (2006)

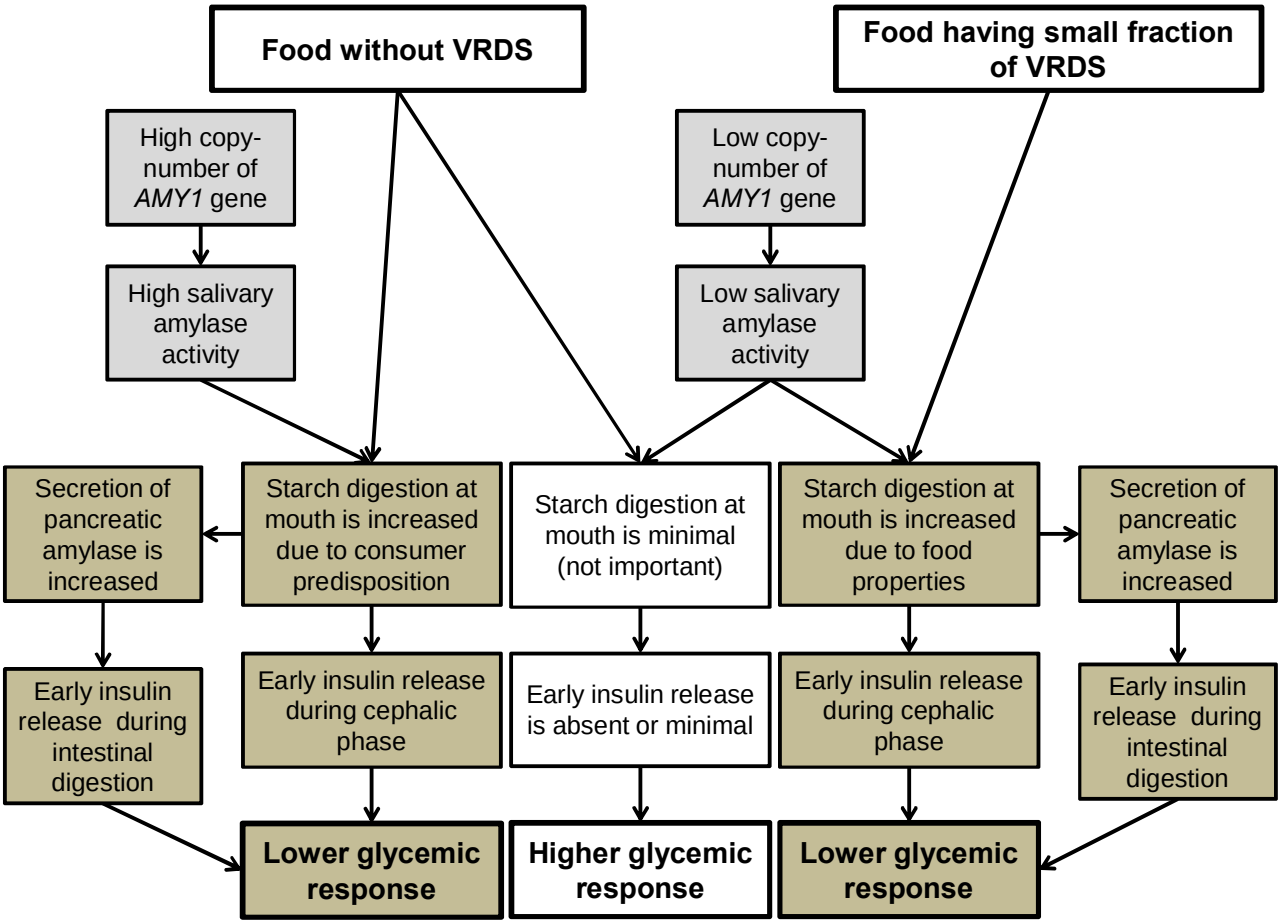

Figure 1. Suggested relationship among food digestibility, copy-number of *AMY1* gene, and glycemic response. Note that food digestibility is affected by food microstructure, which in turn is determinate by food composition and processing. VRDS: Very rapidly digestible starch.