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Starch-Guest Inclusion Complexes: Formation, Structure, and Enzymatic Digestion

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Abstract

Starch/amylose-guest inclusion complexes, a class of supramolecular host-guest assemblies, are of critical importance in the processing, preservation, digestion, nutrients/energy uptake, and health outcomes of starch-containing foods. Particularly, the formation of inclusion complex has been suggested to lower the rate and extent of enzymatic digestion of starch and starch-containing foods. Compared with rapidly digestible starch, starch inclusion complex may fall into the category of slowly digestible starch, providing sustained glucose release and maintaining glucose homeostasis. Therefore, the ability of starch-guest inclusion complex to alter the digestive behavior of energy-dense starchy foods has been of interest to many researchers and has the potential to be developed and formulated into functional foods. In this article, we provide a comprehensive and critical review on the current knowledge of the *in vitro* and *in vivo* enzymatic digestion of starch-guest inclusion complexes, by emphasizing the structure-digestibility relationship. We examine the preparation methods employed, crystalline structures obtained, and physicochemical properties characterized in previous reports, which all have implications on the digestive behavior reported on the starch-guest inclusion complexes. In addition, we give suggestions on future research to elucidate the digestive properties of starch-guest inclusion complexes and to develop functional structures based on these complexes for use in foods and nutrition.

Keywords: starch inclusion complex; amylose inclusion complex; V-type amylose; enzymatic digestion; slowly digestible starch

1. Introduction

The linear component of starch, including largely amylose and some linear segments of amylopectin side chains, can form non-covalent inclusion complexes with a wide variety of small molecules in foods (Figure 1), e.g., iodine (Bluhm and Zugenmaier 1981; Mottiar and Altosaar 2011), alcohols (Nishiyama et al. 2010), fatty acids and their esters (Godet et al. 1993; Lay Ma, Floros, and Ziegler 2011), emulsifiers (Kong, Bhosale, and Ziegler 2018), and aroma compounds (Tapanapunnitikul et al. 2007; Conde-Petit, Escher, and Nuessli 2006). When forming the inclusion complexes, amylose exists as left-handed single helices with a hydrophilic outer surface and a hydrophobic helical channel that accommodates the guest molecules (Immel and Lichtenthaler 2000). Amylose-guest inclusion complexes have great impact on the processing, preservation, digestion, nutrients/energy uptake, and health outcomes of starchy foods (Putseys, Lamberts, and Delcour 2010). For example, the formation of amylose-iodine inclusion complex is a simple and fast method to test the presence of starch by showing a purple-black color. The formation of starch-emulsifier inclusion complexes, e.g., amylose-glycerol monostearate, is thought to be responsible for the retardation of starch retrogradation and the antistaling effects of such emulsifiers in baked foods (Stauffer 1995). In addition, starch may sequester desirable aroma compounds and thus suppress their impact. Starch may, on the other hand, refrain off-flavors or their precursors, for instance, ferulic acid in whole grain breads (Wang et al. 2011), and improve bread quality.

The nutritional importance of starch-guest inclusion complex involves its influence on the digestibility of starch in starch-containing foods. This is largely associated with the significant changes in the structure and properties of starch when complexed with guest materials, including decreased solubility, increased gelatinization temperature (Eliasson, Carlson, and Larsson 1981)

and retarded retrogradation during storage (Krog 1971). Due to these changes, the enzymatic digestion of starch could be inhibited. Extensive studies have shown that the consumption of rapidly digestible starchy food causes postprandial hyperglycemia and hyperinsulinemia cycle, which may lead to metabolic diseases including metabolic syndrome, obesity, type 2 diabetes, and cardiovascular diseases (Byrnes, Miller, and Denyer 1995; Ludwig 2002). The retardation of starch digestion by forming inclusion complexes has attracted much attention because this structure may not only imply a novel physicochemical route to alter the digestive behavior of energy-dense starchy foods, but also deliver bioactive compounds of interest to the lower gastrointestinal tract in a targeting and/or controlled manner.

There are several reviews on the formation, properties, and functionalities of amylose inclusion complexes (Obiro, Sinha Ray, and Emmambux 2012; Putseys, Lamberts, and Delcour 2010; Feng et al. 2011), but discrepancies, contradictory findings, and ambiguous statements remain on the digestibility and nutrition of starch-guest inclusion complexes. In this review article, we will start off with a brief introduction of the formation and structure of amylose inclusion complex, which will be frequently referred to in the following discussion. Then, we will comprehensively and critically review the digestibility of starch-guest inclusion complexes, elaborating both evidences and unknowns, both conclusions and uncertainties, and both merits and shortcomings in previous reports. Based on that, we will propose future research strategies on elucidating the digestive properties of starch-guest inclusion complexes and on developing functional structures of complexes that can be used in food and nutrition.

2. Formation

Starch represents the most complex molecular organization of biological molecules and comprises of multiple levels of structure. In a widely accepted structural model of starch (Buléon

et al. 1998), the crystalline phases were formed by regularly arranged double helices of adjacent amylopectin side chains, whereas amylose molecules reside in the amorphous regions. These amylose molecules are mostly non-crystalline, and may also complex with endogenous lipids especially in starches of certain botanical origin, e.g., potato starch. However, their existence, if any, would be in a small amount and thus difficult to detect. During food processing, especially upon starch gelatinization, starch-lipid inclusion complexes could form because amylose molecules are released from the gelatinized starch granules and the increased molecular mobility facilitates their interaction with lipids or other food components. During the cooling of starch-containing foods, starch inclusion complexation competes with starch retrogradation. If guest molecules are available in the proximity of amylose or amylopectin, inclusion complexes can be formed and impede the progression of retrogradation. When starch is consumed and digested through the digestive tract, linear segments of the polysaccharide may interact with other food components, e.g., lipids, and form inclusion complexes (Larsson and Miezis 1979). Described above are cases where starch-guest inclusion complexes can be formed in a relatively small scale. Some of these cases are hypothesized but difficult to test, while some others may have a greater impact on food quality and digestion.

In studies on the structural details and physicochemical properties of starch-guest inclusion complexes, researchers prepare the inclusion complexes using well-defined raw materials, i.e., starch/amylose and guests of interest. Reported methods to produce starch-guest inclusion complexes can be categorized into four groups, namely dimethyl sulfoxide (DMSO) method, alkali method, high temperature-water method, and pre-formed "empty" helix method (Kong and Ziegler 2014b) (Figure 2). The first three methods require that starch is "destructured" and amylose is converted to random coils before guest compounds are introduced. The coil to helix transition is

thus induced by changing solvent quality, including composition and temperature, such that the hydrophobic interaction between amylose helical interior and guest compounds is favored over the dissolution of the guest in the solvent. In these three methods, starch/amylose can be "destructured" and dissolved into random coils by three solvent systems, i.e, DMSO aqueous solution (with 70% or more DMSO), alkaline solution (for instance, 0.1 M KCl), and superheated water (usually over ~140 °C), respectively. These routes involve high temperature processing, where the guest molecules are necessarily exposed to the same temperatures (over 90 °C in most cases). This may potentially affect the stability of guest compounds, especially those heat-labile ones. The latest strategy in the pre-formed "empty" helix method is to first prepare "empty" amylose helices and then "insert" guest molecules into the helices at temperatures lower than that of "destructuring" the amylose helices (Kong and Ziegler 2014b). This method is simple, scalable and appropriate for heat-labile and expensive compounds, and thus has a great potential for further development.

In the literature, there are other methods reported, e.g., single crystal growth method (Nishiyama et al. 2010), steam jet cooking method (Fanta et al. 2010), and enzymatic synthesis method (Kaneko, Beppu, and Kadokawa 2008). Yet, inclusion complexes made by these methods were not used in digestion studies.

3. Microstructure

When complexed with guest molecules, amylose forms a left-handed single helical structure wrapping the guest molecule. The helices may exist in an amorphous phase, as suggested by several *in vitro* (Biliaderis and Galloway 1989) and *in vivo* (Holm et al. 1983) formation studies. The helices, in most other cases, tend to stack together in a crystalline structure known as the V-type structure. This crystalline structure is usually identified by the X-ray diffraction (XRD) technique. Depending on the size and shape of the guest molecules, a series of V subtypes could

be formed (Figure 3). With small guest molecules, e.g., iodine (Bluhm and Zugenmaier 1981) and fatty acids (Godet et al. 1993), amylose inclusion complex can be in the forms of V_6-hydrate (V_{6h}) or V_6-anhydrous (V_{6a}). The V_{6h} form has a hexagonal unit cell with parameters a = b = 13.65 Å, and c = 8.05 Å (Brisson, Chanzy, and Winter 1991). Upon losing water from within the unit cell, the V_{6h} form can shrink to V_{6a}, which has an orthorhombic unit cell structure with dimensions of approximately a = 13.0 Å, b = 23.0 Å, and c = 8.05 Å (Rundle 1947; Winter and Sarko 1974; Zobel, French, and Hinkle 1967). When molecules bulkier than linear alcohols are included into the amylose helical cavity, crystals of larger dimensions can be obtained, e.g., the amylose-*tert*-butanol inclusion complex ($V_{tert\text{-butanol}}$, or V_7) containing 7-fold single helices with larger cavities to accommodate the guest molecules (Zaslow 1963). A number of guest molecules, e.g., 2-propanol (Nishiyama et al. 2010), n-butyric acid (Takeo, Tokumura, and Kuge 1973), cyclohexanol (Yamashita, Ryugo, and Monobe 1973), menthone (Tapanapunnitikul et al. 2007; Nuessli et al. 2003), thymol (Tapanapunnitikul et al. 2007), fenchone (Nuessli et al. 2003), geraniol (Nuessli et al. 2003), salicylic acid (Oguchi et al. 1998), and 2-naphthol (Uchino et al. 2001), can form V_7 type inclusion complexes with amylose. A few molecules, e.g., 1-naphthol (Yamashita, Ryugo, and Monobe 1973; Cardoso et al. 2007), quinolone (Yamashita, Ryugo, and Monobe 1973), and salicylic acid and its analogues (Uchino et al. 2002), are able to induce amylose inclusion complexes with 8-fold helices (V_8). Described above is a classification of amylose inclusion complexes depending on the number of glucopyranosyl units per turn in the helices. Under each category, different guest molecules may induce small variations in helical packing and distortion of the crystalline region (Nuessli et al. 2003; Helbert and Chanzy 1994; Sarko and Zugenmaier 1980). Recently, linear fatty acids were shown to form different V_6 types and even V_7 type

153 inclusion complexes with amylose, depending on crystallization conditions during crystal growth
154 (Le et al. 2016; Le et al. 2018).

155 **4. *In vitro* digestibility**

156 It has long been noticed of the significance of naturally occurring lipid components in starch
157 granules in altering starch properties, including its enzymatic digestibility. Initially, it was
158 suggested that the influence could be partially due to the formation of amylose-lipid inclusion
159 complexes, and partially because lipids could form a membrane at the periphery of the granules
160 that limits enzymatic digestion of starch (Hanna and Lelievre 1975). By adding surfactants to
161 disrupt the lipid membrane possibly formed (Stoeckenius and Engelman 1969), the retardation in
162 α- and β-amylase digestion of amylose was confirmed to be attributed to the amylose inclusion
163 complexes formed with native lipids, and/or sodium lauryl sulfate and lysophosphatidylcholine
164 (LPC) (Hanna and Lelievre 1975). Additionally, amylose-surfactant inclusion complexes were
165 shown to significantly impede enzymatic digestion of amylose by β-amylase, evidenced from
166 decreased maltose production (Kim and Robinson 1979). The complexes were found to be partially
167 degraded and the degree of digestion varied with different surfactants.

168 Given profound influence of amylose inclusion complexes on starch digestion,
169 starch/amylose-guest inclusion complexes were prepared and subjected to enzymatic digestion
170 studies. Comparing with free amylose, Holm et al. found that amylose-oleic acid and amylose-
171 LPC inclusion complexes showed a reduced susceptibility to hog pancreatic α-amylase (Figure 4A)
172 (Holm et al. 1983). Both free and complexed amylose, however, could be totally degraded in the
173 presence of excess α-amylase and given sufficient time. In other studies, starches of various
174 botanical origins, e.g., wheat starch (Figure 4B & 4C) (Hanna and Lelievre 1975; Wang et al. 2016;
175 Ahmadi-Abhari, Woortman, Hamer, et al. 2013; Ahmadi-Abhari, Woortman, Oudhuis, et al. 2013),

potato starch (Kawai et al. 2012), high amylose maize starch (Foucault et al. 2016), sago starch (Cui and Oates 1999), etc., were used to complex with lipids, including free fatty acids, monoglycerides, and LPC. The effect of chain length of fatty acids and monoglycerides on the structure and digestion of starch inclusion complex will be discussed later in this review. The starch-LPC inclusion complex has been investigated by a number of researchers, e.g., wheat starch-LPC inclusion complex (Ahmadi-Abhari, Woortman, Hamer, et al. 2013; Ahmadi-Abhari, Woortman, Oudhuis, et al. 2013). The wheat starch-LPC inclusion complex was formed by heating at 95 °C using a rapid visco analyzer and then subjected to α-amylase digestion at 37 °C. Higher LPC concentration resulted in a lower enzymatic susceptibility of wheat starch. Cui and Oates used sago starch to complex with LPC and other lipids (Cui and Oates 1999). Since a lower complexation temperature at 60 °C was used, the ability to form inclusion complex was limited, as evidenced from relatively larger gelatinization endotherms than inclusion complex endotherms on differential scanning calorimetry (DSC) thermograms. Yet, a reduced enzyme susceptibility of starch in the presence of lipids was found, which was still encouraging. Limited inclusion complexation was also seen in a few other reports (Kawai et al. 2012; Wang et al. 2016), where no significant difference in starch digestibility compared to control samples was observed. These studies used temperatures that were not sufficiently high to “destructure” starch granules and release starch molecules for inclusion complexation with guest molecules. While most studies used native starch and isolated amylose with unknown degree of polymerization (DP) and polydispersity, the effect of amylose chain length (DP) on inclusion complex formation and subsequent enzymatic digestion was investigated (Gelders et al. 2005). An increase in amylose DP from 60 to 950 could slow down enzymatic digestion of the inclusion complex formed, possibly due to greater lamella thickness formed by amylose with higher DP.

In several other studies, researchers prepared starch/amylose-guest inclusion complexes for purposes other than studying their enzymatic digestion, but their findings may also be useful for understanding the digestion behavior. For instance, *in vitro* digestion was employed to study crystal structures of the V-type amylose inclusion complexes by enzymatically hydrolyzing the amorphous regions (Figure 4E) (Seneviratne and Biliaderis 1991; Godet et al. 1996). These methods were aligned with the idea that enzymatic hydrolysis would initially take place in the amorphous regions (Jane and Robyt 1984), including the amorphous growth rings and amorphous lamellae.

In addition, a few other researchers focused on the release properties of guest compounds triggered and controlled by enzymatic hydrolysis of starch/amylose-guest inclusion complexes. Lesmes et al. produced starch-stearic acid inclusion complex and tested it for controlled release upon pancreatic amylase (α-amylase) treatment at 37 °C for 24 h (Lesmes, Barchechath, and Shimoni 2008). The stearic acid released from high amylose corn starch matrix was significantly lesser than that from normal corn starch, suggesting high amylose starch more efficiently formed inclusion complex and impeded starch degradation to release the guest fatty acid. The same group of researchers further investigated the effect of fatty acid unsaturation on the release properties and found that the extent of guest release was inversely related to the degree of unsaturation in fatty acids (Lesmes et al. 2009). The existence of double bonds in *cis* conformation could distort amylose helices and result in less closely packed crystalline phase. Therefore, amylose inclusion complex with unsaturated fatty acids could be digested faster and to a greater extent than that with saturated fatty acids. For the same purpose of controlling guest release, polyunsaturated fatty acids were encapsulated by spring dextrin, which can be considered as shorter amylose chains produced from starch (Xu et al. 2013). The release of complexed α-linolenic acid and linoleic acid was

triggered by α-amylase, but the digestion rates were not compared with that of uncomplexed spring dextrin. In these studies, although there could be a lack of a comprehensive investigation of release kinetics, the starch-guest inclusion complex showed to be a promising delivery tool due to its resistance to enzymatic hydrolysis.

5. *In vivo* digestibility

As introduced earlier, amylose-guest inclusion complexation could take place *in situ* in the digestive tract, where linear glucan fragments from partially digested amylose and amylopectin could complex with free fatty acids released from lipid digestion (Larsson and Miezis 1979). However, this phenomenon was difficult to directly characterize and monitor, therefore, few studies paid attention to the formation of amylose inclusion complex during digestion.

So far, there have not been extensive studies on the *in vivo* digestibility of starch-guest inclusion complex. Back in the 1980s, Björck et al. (1984) found drum-dried wheat flour, which contained much higher amount of amylose-lipid inclusion complex than untreated and boiled wheat flour, was not only less susceptible to salivary amylase hydrolysis, but also gave a lower plasma glucose response and delayed insulin response in rats (Figure 5A). However, Holm et al. (1983) did not notice significant difference in plasma glucose and insulin response between rats fed with amylose and amylose-lipid inclusion complex (Figure 5B). This could be explained by the low substrate concentration used in the latter experiment, which will also be discussed later in this review.

Relatively recent studies on starch inclusion complex digestion extended to larger animals and human subjects. Murray et al. (1998) compared the digestibility of starch-lipid inclusion complex with that of maltodextrin and resistant starch in ileal-cannulated dogs. The extent of digestion and fecal bulking capacity of starch inclusion complex were both intermediate between the control and

resistant starch, marking it as a slowly digestible starch. Hasjim et al. (2010) investigated the effect of high amylose maize starch-palmitic acid inclusion complex on the plasma glucose and insulin responses in human subjects. Postprandial plasma-glucose and insulin responses (measured as both peak maxima and average areas under curve) in subjects given bread formulated with starch inclusion complex were only about half of those responses in subjects having consumed control white bread (Figure 5C). Similar experiments were carried out by (Lau, Zhou, and Henry 2016), where bread samples containing starch inclusion complexes with different types of fats/oils were consumed by human subjects, yet mixed results were obtained. There was a significant glucose response between test bread and control, while insulin response was not altered by adding starch-lipid inclusion complexes (Figure 5 D). Although studies on *in vivo* digestion of starch-guest inclusion complex are few, the available results so far are inspiring.

6. Structure-digestibility relationship

To summarize the previous *in vitro* and *in vivo* studies listed above, many found that inclusion complexation with guest compounds, especially lipids, could inhibit the enzymatic digestion of starch or amylose, however, discrepancies did exist. The causes of experimental discrepancies can be multifaceted.

First, the formation of amylose/starch-guest inclusion complex needs to be well characterized and interpreted. Starches of various botanical origins and different types of guest compounds (mostly lipids) were investigated in combinations. The starches vary in their ratios of amylose to amylopectin components and length of polymer chains (DP). Lipids, including free fatty acids, monoglycerides, and LPC, differ in their structures, e.g., number of fatty acid chains and fatty acid chain length. Given these variations, it appears that the structure of amylose-guest inclusion complex has not been paid sufficient attention to in some of the studies on their enzymatic

digestion. Owing to the advances in characterization technologies, the existence of amylose-guest inclusion complex can be evidenced by a number of complementary techniques, i.e., DSC (Karkalas et al. 1995; Biliaderis, Page, and Maurice 1986), XRD (Godet et al. 1993; Le Bail et al. 1999), nuclear magnetic resonance (NMR) (Lebail et al. 2000), Fourier transform infrared spectroscopy (FTIR) (Lay Ma, Floros, and Ziegler 2011; Kong and Ziegler 2014a), and to some extent small-angle X-ray scattering (SAXS) (Putseys et al. 2011), transmission electron microscopy (TEM) (Godet et al. 1996), Raman spectroscopy (Carlson et al. 1979), and electron paramagnetic resonance (EPR) spectroscopy (Kong et al. 2018). The successful formation of amylose/starch inclusion complex usually requires evidence from more than one techniques. For instance, DSC, XRD, and FTIR were used in combination to provide thermal, structural and compositional evidences of inclusion complex formation (Lay Ma, Floros, and Ziegler 2011; Kong, Bhosale, and Ziegler 2018). Without such prior knowledge on the structure of inclusion complex formed, the retardation effect of inclusion complex on starch digestion rate could not be properly justified.

As discussed earlier, limited inclusion complexation has been seen in a number of studies on the enzymatic digestion of starch inclusion complex, and was mainly attributed to ineffective formation methods. For instance, by reacting lipids with sago starch at around gelatinization temperature, i.e., 60 °C, the DSC thermograms actually displayed only a small portion of inclusion complex formed compared with much larger gelatinization endotherms of ungelatinized starch portions (Cui and Oates 1999). Even though, reduced enzyme susceptibility was noticed and the extent of enzyme susceptibility was found to be reversely associated with complex-forming ability of lipids. In several other studies where only little or negligible amount of inclusion complex was

290 formed, it was not surprising that enzymatic digestibility was not significantly altered (Kawai et
291 al. 2012; Wang et al. 2016; Kim et al. 2017).

292 As another example, despite profound reductions in postprandial plasma-glucose and insulin
293 responses found by Hasjim et al. (2010), it was debatable how effective the starch-palmitic acid
294 inclusion complex was in this study. Given the absence of starch-palmitic acid endotherm during
295 the first scan on DSC thermogram, the inclusion complex preparation method might not be
296 effective. A greater amount of starch-palmitic acid inclusion complex was actually formed during
297 rescanning of the samples, which is a process similar to the high temperature method for starch
298 inclusion complex formation during cooling. Guest compounds could induce inclusion
299 complexation with amylose/starch molecules that were converted to loose helical or random coil
300 conformations above 140 – 160 °C (Creek, Ziegler, and Runt 2006), upon first heating. Therefore,
301 the difference in plasma-glucose and insulin responses could simply be a result of using high
302 amylose starch to replace wheat starch component in flour, since high amylose starch itself could
303 result in a significantly lower glucose and insulin responses than amylopectin (Behall, Scholfield,
304 and Canary 1988).

305 Secondly, the resistance to enzymatic digestion is dependent on the structural organization of
306 helical starch inclusion complex, which can be represented by crystallinity and thermal stability.
307 The structure and stability of the amylose inclusion complex vary with guest compounds and
308 formation methods. Guest compounds of different sizes and shapes may induce different
309 crystalline structures and thermal stability. Lipids are the most used guest compounds in studying
310 the digestion of starch-guest inclusion complex. The fatty acid chain length, degree of unsaturation,
311 and identity of the polar head of the lipids all impact the inclusion complex properties (Putseys,
312 Lamberts, and Delcour 2010). Lipids with longer hydrocarbon chains allow for more hydrophobic

interactions with the hydrophobic helix cavity of amylose, and thus result in higher thermal stability (Tufvesson, Wahlgren, and Eliasson 2003). The presence of carbon-carbon double bond, especially in *cis* configuration, may interfere with amylose wrapping around the alkyl chain and result in distorted helical element or partial complexation with lower stability (Lalush et al. 2004). Functional groups at the end of lipid hydrocarbon chains have a great impact on the localization of guest lipids and the crystallization of adjacent helices (Kong et al. 2018; Kong, Perez-Santos, and Ziegler 2018). A larger head group has been proposed to impede crystallization and crystal growth, and may result in lower crystallinity and lower thermal stability (Lay Ma, Floros, and Ziegler 2011). Hence, the intermolecular association and crystalline structure affect thermal stability of the inclusion complex, and may also determine the stability of inclusion complex during enzymatic digestion.

Czuchajowska, Sievert, and Pomeranz (1991) found that amylose-lipid inclusion complex formation was favored over amylose retrogradation when effective complexing lipids were used, i.e., LPC and sodium stearoyl lactylate (SSL). But hydroxylated lecithin (OHL) was not as effective as LPC and SSL in inclusion complexation as well as inhibiting amylose retrogradation. From the structural point of view, the bulky head groups of OHL including hydroxylated fatty acid, choline and phosphate groups on the glycerol created too much stearic hindrance for inserting the hydrocarbon chains into amylose helices. Both LPC and SSL have one less fatty acid chain than OHL on the glycerol molecule and thus were able to form inclusion complex more efficiently.

Crowe, Seligman, and Copeland (2000) found that enzymatic hydrolysis of amylose was retarded by 35% ($P < 0.05$) after complexation with free fatty acids, including lauric, myristic, palmitic, and oleic acids, and LPC, compared with uncomplexed amylose after 2 h digestion at 37 °C. Yet the fatty acids had no effect on the digestion of amylopectin. However, the initial rates

of hydrolysis (within 30 min) were almost identical for the complexed and uncomplexed amylose, whereas Holm et al. found that the complexed amylose was hydrolyzed at a significantly lower rate than the uncomplexed sample during the whole experiment period (Holm et al. 1983). It implies probable structural differences between the inclusion complex samples used in the two studies; the inclusion complex formed under simulated physiological conditions (37 °C) could result in more amorphous region (less crystallinity) than the alkali neutralization method, and thus showed similar susceptibility to enzymes as uncomplexed amylose at initial hydrolysis.

As discussed earlier, the variations in structural organization can be induced by lipid guests of varying chain length (Biliaderis, Page, and Maurice 1986), which include an alternative structural difference, i.e., the so-called Type I (Form I) and Type II (Form II) amylose inclusion complexes (Biliaderis and Galloway 1989). Seneviratne and Biliaderis (1991) investigated the α-amylase hydrolysis of amylose-glycerol monostearate inclusion complexes with different degree of structural order, including the type I, type IIa and type IIb. It was found that the rate and the extent of enzymatic degradation were negatively associated with the crystallinity and thermal stability of the inclusion complexes; inclusion complexes with greater crystallinity and higher melting temperature were more resistant to enzymatic degradation. During enzymatic hydrolysis, the crystallinity of enzyme-resistant fractions was not significantly altered, whereas their thermal stability was affected, showing decreasing dissociation temperature and enthalpy. It could suggest that the enzyme first attacked the uncomplexed amylose chains, followed by non-crystalline amylose inclusion complexes (type I). Therefore, similar to mild acid hydrolysis (Biais et al. 2006), enzymatic hydrolysis could initially increase the order and stability of amylose inclusion complex by removing less ordered regions. The α-amylase did hydrolyze highly ordered domains of amylose inclusion complex (type II) because the sample was fully digested at high enzyme levels.

Last but not least, digestion kinetics depends on the experimental conditions, including types of enzyme, concentrations, temperature and time, and animal models in *in vivo* studies. For example, both uncomplexed amylose and amylose-lysolecithin inclusion complex were totally hydrolyzed at quite similar rates within 2 h in the gastrointestinal tract of rat (Holm et al. 1983). In contrast, Murray et al. (1998) reported a significantly lower digestibility of starch inclusion complex and resistant starch than uncomplexed starch in illeal-cannulated dogs. The discrepancy was probably due to differences in sample preparation methods, weight ratios of sample intake, and of course physiological systems in different animals. In the experiment carried out by Murray et al., the average carbohydrate intake in the forms of either uncomplexed or complexed starch was roughly 1% (w/w) of the weight of the dogs, while the rats in Holm et al.'s report were fed with only 0.01% (w/w) of the weight of the rats. This could result in different substrate concentration in the gastrointestinal tract available for enzymatic hydrolysis. Indeed, Holm et al. (1983) found that the hydrolysis of amylose inclusion complex was significantly accelerated by adding excess enzyme.

7. Resistant starch

There are occasions where amylose inclusion complexes were regarded as a type of resistant starch (Hasjim et al. 2010), even in relatively recent reviews (Panyoo and Emmambux 2017), however, most of other researchers did not agree. Although the starch-lipid inclusion complexes can decrease the susceptibility of starch to amylolysis, they have not been regarded as a fraction of resistant starch (Sievert and Pomeranz 1989,1990; Siljeström, Eliasson, and Björck 1989). Indeed, there may be a competition between amylose-lipid inclusion complexation and amylose retrogradation into resistant starch fraction (Sarko and Wu 1978).

By definition, resistant starch is starch or a fraction of starch that is not hydrolyzed by enzymes in the digestive tract, but it is fermented by the gut microflora in the colon (Englyst and Macfarlane 1986; Wyatt and Horn 1988). Resistant starch is generally considered as a type of dietary fiber. Experimentally, it refers to the remaining portion of starch after exhaustive enzymatic hydrolysis. The methods to determine resistant starch contents have been evolving during the last few decades. The type of enzymes used, enzyme concentration, and incubation pH, time and temperature are among the modifications in various reported methods. In a widely used method developed by Megazyme and accepted as AOAC Official Method 2002.02 (AOAC International 2012) and AACC Method 32-40.01 (AACC International 1999), α-amylase and amyloglucosidase were used to hydrolyze samples for 15 h at 37 °C. The residual starch portion is the resistant starch to be determined.

The majority of the studies reviewed here did not adopt the resistant starch assay method in their *in vitro* enzymatic digestion analyses of starch-guest inclusion complex. Therefore, it created uncertainty whether at least a portion of the starch inclusion complex can be regarded as resistant starch. However, an incubation time of 15 h suffices for the adequate time needed for complete digestion as suggested by (Holm et al. 1983). This incubation time of 15 h is definitely longer than the transition time for starch inclusion complex to reach the large intestine, which is usually considered to be around 120 min (Holm et al. 1983). Given the dynamic nature of digestion and the differences in *in vitro* and *in vivo* conditions, it cannot be excluded that some starch inclusion complex may be determined as non-resistant starch by assay, but may enter the large intestine and function as resistant starch.

8. Conclusions

In conclusion, it has been observed in many studies that amylose/starch-guest inclusion complex formation can inhibit the enzymatic digestion of starch or amylose. It is important to understand that the extent of the inhibition effect is determined by the structure of the inclusion complex. In order to obtain a better understanding of the enzymatic digestion behavior of starch-guest inclusion complex and develop functional food ingredients based on starch-guest inclusion complex, we provide the following suggestions for future research on this topic. First, the preparation procedure needs to be well-defined and the starch/amylose-guest inclusion complexes formed should be well-characterized, so that their structure-property relationship can be well-established. This practice will also be beneficial to find the type of starch-lipid inclusion complex that could have the most profound effect on the inhibition of starch digestion. Secondly, official methods and simulated physiological conditions can be used to determine the RS proportion of starch inclusion complex in assays and in reality. Finally, functional or bioactive guest compounds can be incorporated into starch inclusion complex as a way to study the release properties of the guest compounds along with the investigation of their enzymatic digestion.

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