High-Amylose Starches to Bridge the "Fiber Gap": Development, Structure, and Nutritional Functionality

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Abstract: Although high-amylose starches are not a recent innovation, their popularity in recent years has been increasing due to their unique functional properties and enhanced nutritional values in food applications. While high-amylose maize, barley, and potato are commercially available, high-amylose variants of other main crops such as wheat and rice have once been developed more recently and will be available commercially in the near future. This review summarizes the development, structure, and nutritional functionality of high-amylose starches developed and reported so far. The range of biotechnological strategies utilized are reviewed, as are the consequent effects on structural properties at different length scales, as well as sensory aspects of foods containing high-amylose starch (HAS). This review identifies the molecular and microstructural features contributing to digestive enzyme resistance not only in native HAS but also in forms of relevance to food processing. During heat treatment, HAS tends to retain or form dense molecular structures that resist amylase degradation through the retention of the granular structure as well as helices (type-2 resistant starch [RS]), reassociation of glucan chains (type-3 RS), and formation of lipid–amylose complexes (type-5 RS). The review also identifies opportunities for food manufacturers and consumers to incorporate HAS in food products and diets for better nutritional outcomes.

Keywords: amylose, dietary fiber, resistant starch, starch biosynthesis, wheat

Introduction

Starch is the major storage carbohydrate in plants. Its biosynthesis occurs in seeds, tubers, fruits, roots, and leaves. Starch plays important roles not only in the life cycle of plants, but also in human and animal nutrition. The degradation of starch provides much of the energy needed by humans. However, the process is also responsible for undesirable body responses or metabolic disorders, such as excessive energy intake and high blood glucose level. These consequences can become major risk factors resulting in increasingly common diseases such as obesity and type 2 diabetes.

Postprandial metabolism depends on how starch, after ingestion, is digested into glucose that is absorbed in the small intestine. The digestion is initiated by salivary α-amylase, followed by pancreatic α-amylase with smaller oligomers and α-limit dextrins as products. These are further degraded by the small intestinal brush border glucoamylases (Nichols et al., 2003) into glucose as the end product. The starch fraction that escapes the hydrolysis of these enzymes in the small intestine is termed as resistant starch (RS). The health benefits of RS are not limited to reducing risks of metabolic disorders. Its interactions with the microbiota

in the large intestine through fermentation have additional positive effects on the body, including improvement of immune responsiveness and management of colonic diseases (Shanahan, van Sinderen, O'Toole, & Stanton, 2017; Topping & Clifton, 2001; Valentina & Fredrik, 2012).

The underlying mechanism of starch digestion has been classified into two groups: barriers to the access of enzyme to starch and starch structural features controlling the enzyme action once bound to starch (Dhital, Warren, Butterworth, Ellis, & Gidley, 2017). Starch contains two groups of glucan polymers, amylopectin, which is highly branched and has many short branches, and amylose that has a small number of long-chain branches. The amylose content has been shown to have significant correlation with lower digestibility or higher RS content (Li, Jiang, Campbell, Blanco, & Jane, 2008; Regina et al., 2012; Witt, Gidley, & Gilbert, 2010; Zhu, Liu, Wilson, Gu, & Shi, 2011). Although physical and chemical techniques can modify the starch properties for lower susceptibility to amylolysis, the degree of modification through physical techniques is limited and products of chemical modification without clean labelling are not always acceptable to consumers. Thus, biological approaches to increase the amylose content are of current interest.

Breeding high-amylose cereals started in the 1940s (Table 1). Nowadays, a better understanding of how the starch biosynthesis enzymatic machinery functions and the identification of genes related to the biosynthesis of the glucan polymers has enabled

Table 1–History of high-amylose maize starch breeding.

Date	Event	Mutant	Enzyme[a]	Amylose content
1940s	Initiation of the first high-amylose breeding program	*dull* (*du*), *Sugary1* (*su1*)	SSII	65%
1952	Discovery of the *amylose-extender* (*ae*) gene	*ae*	SBEIIb	55%
1958	Commercialization of first high-amylose maize hybrids	*ae*	SBEIIb	55-60%

[a] Enzyme activity down-regulated in mutants (Hallauer, 2001).
SS: starch synthase; SBE: starch branching enzyme.

the manipulation of amylose content in a wide range of crops through traditional breeding or transgenic modification. This has enriched the choice of available varieties with starch that can be tested for desired functional properties in food applications and nutritional functions. However, alterations in amylose content, by whatever means, may affect different levels of starch structure with a whole cascade of functional and nutritional consequences. Thus, it is timely to review these advances together to interconnect selection of genes to be modified through starch digestion into glucose in humans. The current review critically evaluates (1) the effect of approaches to elevating amylose content on molecular and supramolecular features of starch, (2) how these features impact on RS formation associated with food processing and gastrointestinal digestion, and (3) the opportunities for food manufacturers and consumers to incorporate HAS in food products and diets for better nutritional outcomes.

Structural Features of High-Amylose Starch
Overview of high-amylose starch

Normal or wild-type starches consist of two types of glucose polymers, amylose (approximately 25%) and amylopectin (approximately 75%), with traces of lipids and proteins. The linear backbone is α-(1-4)-linked glucan, while branches are linked by α-(1-6) glycosidic bonds. Amylose is predominately linear with rare branches, while amylopectin is composed of about 5% of branch points. Compared to amylose with moderate molecular weight (approximately 10^6 Daltons), amylopectin is known as one of the largest biopolymers and has a much higher molecular weight (approximately 10^8 Daltons). Starch with elevated levels of amylose, compared to the typical wild-type lines, can be termed high-amylose starch (HAS). So far, HAS types from mutant cereal grains such as wheat, maize, rice, barley, as well as potato tuber have been developed.

The detailed analysis of molecular structure focuses on the size distribution patterns of both the total size of the molecule and individual branches through size-exclusion chromatography (SEC, also known as gel-permeation chromatography [GPC]) and fluorophore-assisted carbohydrate electrophoresis (FACE). The SEC elution results give the size distributions of whole starch molecules, and of individual chains following enzymatic debranching, and also enable the calculation of amylose content (Vilaplana, Hasjim, & Gilbert, 2012). Iodine colorimetry is another widely used method to determine amylose content. However, iodine colorimetry usually generates overestimated values compared to SEC due to interference by color development from amylopectin–iodine complexes (Fitzgerald et al., 2009; Jane et al., 1999; Kasemsuwan, Jane, Schnable, Stinard, & Robertson, 1995; Vilaplana et al., 2012). This interference cannot be ignored, especially for HAS in which amylopectin can have very long branches, functioning similarly as amylose. Such long amylopectin arises particularly when starch-branching enzymes have been downregulated, as this results in less-branched amylopectin, and is often referred to as intermediate material. Using the ratio of large-to-small fully branched molecules, or long-to-short individual chains

cannot unambiguously classify intermediate amylopectin/amylose into amylopectin or amylose. An experimental two-dimensional (2D) distribution was proposed to address this problem in HAS by Vilaplana, Meng, Hasjim, and Gilbert (2014), which differentiates the intermediate materials in maize HAS (for example, amylopectin with extra-long branches and branched amylose), although it has not yet been applied to HAS from other botanical origins.

Starch structure has been a topic of wide investigation and has been reviewed elsewhere (Wang, Bogracheva, & Hedley, 1998; Zobel, 1988). There have been increasing numbers of studies on the structural features responsible for the unique properties of HAS (for example, maize [Jane et al., 1999; Shi, Capitani, Trzasko, & Jeffcoat, 1998], wheat [Regina et al., 2010], barley [Regina et al., 2012], and rice [Butardo et al., 2017; Wei, Xu, et al., 2010]). The structural features can be categorized into 6 levels (Figure 1): from the lowest level of structure, individual chains, to the highest level of structure, starch deposition in grains (Ball et al., 1996). The change in length of chains can affect the double-helix structure of linear α-(1-4) glucan chains. The packing of helical structure forms a semicrystalline lamellar structure, with a lamellar repeat of 9 to 10 nm within granules, which is almost conserved among all starches in spite of botanical origins and locations. At the next level, starch granules consist of alternating amorphous and crystalline regions, generally defined as "growth rings." It is generally accepted that the amylopectin molecules are radially organized with the reducing ends pointing to the hilum, which is the center of the growth rings. The molecular structure and arrangement (nm level) impact the supramolecular structure (μm level) as well as the granular structure such as surface, size, and shape. The subsequent subsections will elaborate the role of the multilevel structures of HAS on the physicochemical properties.

Individual branches

The branch population of amylopectin has 3 broad classes: A, B, and C as proposed in the generally accepted "cluster model" (Hizukuri, 1986). In each amylopectin molecule, A-chains have themselves no branches but branch out from B-chains. B-chains bind to other B or C-chains. C-chains have the sole reducing end of an amylopectin molecule. The chain-length distribution can be measured by SEC or FACE, after debranching the solubilized clusters into individual chains by isoamylase that cleaves α-(1-6)-linkages (Wu, Li, & Gilbert, 2014). Typically, degrees of polymerization (DPs) of less than 20 are classified as A-chains, but it is emphasized that all A, B, and C chains can have a wide range of DPs. Compared to A-chains, B-chains are longer and consist of 20 to 75 glucose units (Hizukuri, 1986). By comparing amylopectin branches from different botanical origins, Hanashiro, Abe, and Hizukuri (1996) proposed another shorter periodicity in chain length, which can be divided into the fractions of A- (DP 6 to 12), B1- (DP 13 to 24), B2- (DP 25 to 36), and B3-chains (DP >36). As an alternative to the "cluster model," Bertoft (2004) proposed a "building block backbone model" of cluster interconnections, that is, the amylopectin branches are distributed along a
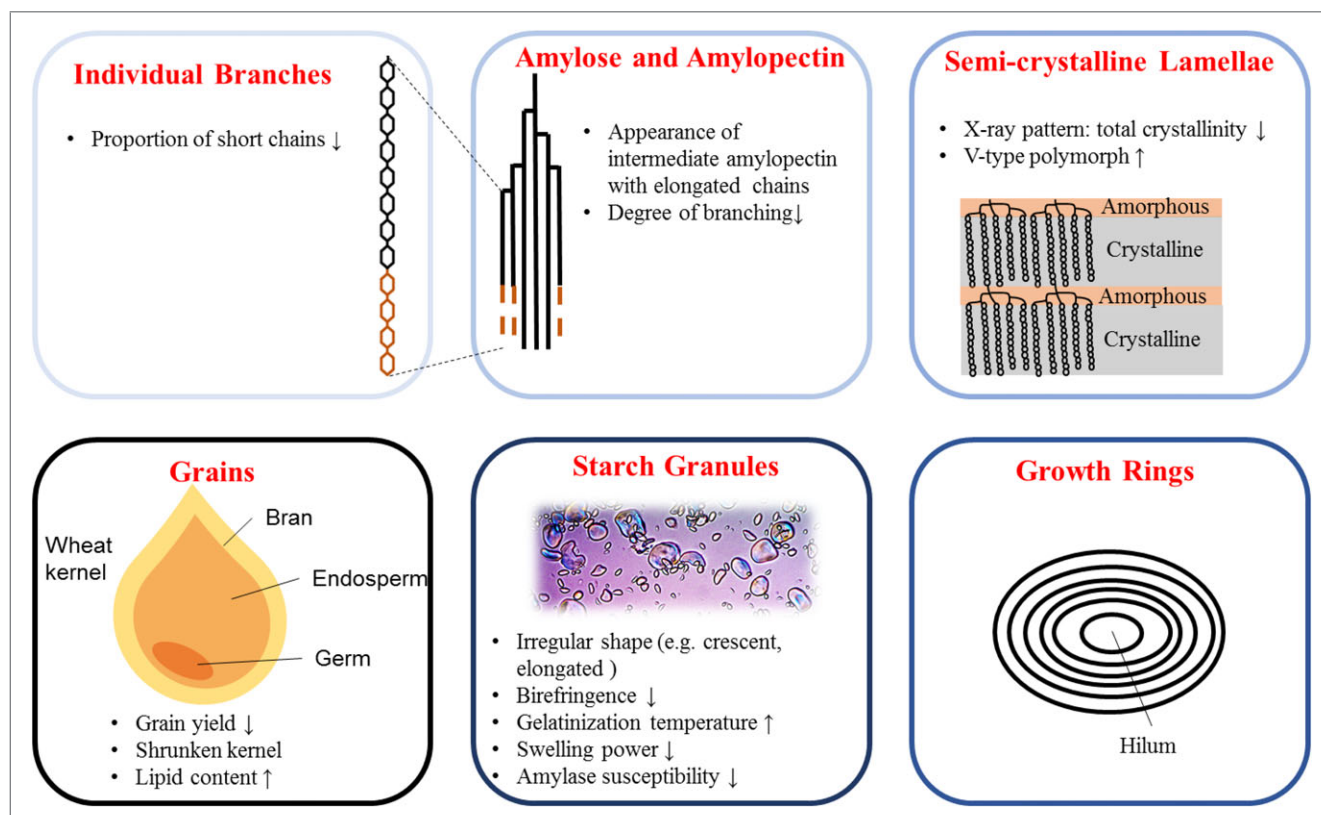
Figure 1–Structural properties of high-amylose starch using wheat as an example. The elongation of individual branches is shown in yellow. (Modified from Dona, Pages, Gilbert, & Kuchel, 2010).

backbone extending in an almost perpendicular direction. More details about the models of amylopectin structure are available elsewhere (Bertoft, 2013, 2017).

As shown in Table 2, HAS from mutant lines with altered expression level of biosynthesis enzymes (starch synthases [SSs] or starch-branching enzymes [SBEs]) generally show an increase in the proportion of relatively long chains (about DP > 30) of certain DP ranges and a decrease in relatively short chains (about 10 < DP < 20), accompanied by an increase in very short chains (about DP < 10). However, the exact range of the branch changes is still ambiguous among the cereal crops, at least in part due to the downregulation level of enzyme expression not being consistent between studies.

Granular distribution of amylopectin and amylose

Although the location of amylose in starch granules is still the topic of debate, in normal raw starch, it is generally accepted that amylose is tangentially located to the radial orientation of amylopectin chains, which minimizes the chances of double-helix formation between the two polymers. The periphery of the starch granule was proposed to contain higher amylose content, compared to the core of the granule (Jane, 2006). The difference was observed through surface chemical gelatinization, which allows the separation of the outer and the inner fractions of starch granules (Jane & Shen, 1993; Pan & Jane, 2000). Amylose may be largely free of interaction with amylopectin as well as other amylose chains in non–high-amylose starches. This hypothesis is supported by the observation that amylopectin retains its crystallinity, while amylose mostly leaches out in the form of a single chain just below gelatinization temperature (Ring, L'Anson,

& Morris, 1985). Amylose and amylopectin in potato may be relatively more separated than in maize (Zobel, 1988). However, the location of amylose in HAS remains to be described. The questions not fully understood include: (1) Do chains of intermediate material in HAS form double helices? (2) Do more amylose chains interact with intermediate amylopectin, and consequently, is less amylose leached out during granule swelling? (3) Do the elongated chains of amylopectin complex with lipids in HAS?

In HAS, it is hypothesized that amylopectin with an increased ratio of longer chains may form radially longer clusters with chains extending through multiple crystalline regions, leading to a more stable lamellar structure (Jane et al., 1999; McPherson & Jane, 1999). This may explain the observation (Butardo et al., 2011, 2017; Li et al., 2008; Regina et al., 2012; Zhu et al., 2011) that short chains may result in imperfections in the formation of crystallites, whereas starch with fewer short chains has better enzymatic resistance and heat stability.

Semicrystalline lamellae (A, B, and V polymorphs)

The packing of double helices of amylopectin chains in HAS tends to result in hexagonal unit cells with B-type crystallinity (X-ray diffraction pattern) (Wang et al., 1998). The wild-type cereal starch generally has monoclinic unit cells and shows an A-type crystallinity pattern, which is relatively more compact and binds with less water. Both crystal polymorphs show characteristic peaks in X-ray diffraction patterns. A main diffraction doublet at 17° and 18° Bragg angles (2θ) are the signature peaks for an A-type crystal pattern. A diffraction singlet at 17° and peaks at approximately 5.5° 2θ are the signature peaks for a B-type crystal pattern. The reason for this polymorphism has been proposed to be that longer

Table 2–Three biological strategies to enhance amylose content.

Biological approaches	Reference	Plant	Methods	Variations in enzyme alleles	Amylose content of wild type	Amylose content of mutated or transformed lines	Changes of amylopectin molecular structure
Suppression of SBE	Shi et al. (1998)	Maize	N/A	SBE II	27%	57%, 71%	DP 6 to 10↓, DP 11 to 20↓, DP >20↑
	Jobling et al. (1999)	Potato	RNA interference	SBE II (A)	30%	39%	DP 6 to 23 ↓, DP 23 to 60 ↑
	Schwall et al. (2000)	Potato	RNA interference	SBE II (A) + I (B)	28%	59% to 75%	DP 6 to 15 ↓, DP 20 to 60 ↑
	Blauth et al. (2002)	Maize	Identification and hybridization	SBE I		similar to wild-type[a]	similar to wild-type
	Satoh et al. (2003)	Rice	Identification	SBE I		similar to wild-type	DP 6 to 10↑, DP 12 to 21↓, DP 24 to 34↑, DP 37 to 60↓
	Regina et al. (2004)	Wheat	Identification and hybridization	SBE I		similar to wild-type	similar to wild-type
	Hofvander, Andersson, Larsson, and Larsson (2004)	Potato	RNA interference	SBE II (A) + I (B)	26% to 28%	54% to 94%	N/A
	Regina et al. (2006)	Wheat	RNA interference	SBE IIa + IIb / SBE IIb	32%	89% / Similar to wild-type	DP 4 to 12↓, DP >12↑ / Similar to wild-type
	Regina et al. (2010)	Barley	RNA interference	SBE IIa + IIb / SBE IIa / SBE IIb	29%	89% / 38% / similar to wild-type	DP 6 to 8↑, DP 9 to 13↓, DP 15 to 30↑ / DP 7 to 9↑, DP 10 to 12↓, DP 14 to 18↑ / DP 7 to 10↑, DP 12 to 18↓
	Butardo et al. (2011)	Rice	RNA interference	SBE IIb	20%	41%	DP 6 to 12 ↓, DP ≥14↑
	Slade et al. (2012)	Wheat	Identification and hybridization	SBE IIa	bread wheat: 23%; durum wheat: 24%	bread wheat: 56%; durum wheat: 47%	N/A
	Carciofi et al. (2012)	Barley	RNA interference	SBE IIa + IIb + I	30%	99%	N/A
	Zhu et al. (2012)	Rice	RNA interference	SBE I + IIb	27%	65%	DP 6 to 23 ↓, DP 24 to 60 ↑
	Regina et al. (2015)	Wheat	Identification and hybridization	SBE IIa + IIb / SBE IIa	30%	85% / 67%	DP 9 to 13↓, DP>24↑; / Similar changes as SBEIIa but DP 14 to 15↓
	Hazard et al. (2015)	Wheat	Identification and hybridization	SBE IIa + IIb	28%	45%	N/A
	Schonhofen et al. (2016)	Wheat	Identification and hybridization	SBE IIa + IIb	31%	50%	N/A

(Continued)

Table 2–Continued.

Biological approaches	Reference	Plant	Methods	Variations in enzyme alleles	Amylose content of wild type	Amylose content of mutated or transformed lines	Changes of amylopectin molecular structure
Suppression of soluble SS	Campbell, White, and Pollak (1994)	Maize	Identification and hybridization	SS II	25%	39%	N/A
	Craig et al. (1998), Wang et al. (1998)	Pea	Identification and hybridization	SS II	Approximately 30%	Approximately 50%	Fewer chains of intermediate length and more very short and very long chains
	A. Edwards et al. (1999)	Potato	RNA interference	SS II	N/A	N/A	DP 6 to 12↑, DP 13 to 35↓
	Yamamori et al. (2000)	Wheat	Identification and hybridization	SS IIa	30%	37%	DP 6 to 10↑, DP 11 to 25↓, DP 29 to 36↑
	Morell et al. (2003)	Barley	Identification	SS IIa	25%	47% to 71%	DP 6 to 11↑, DP 12 to 30↓
	Zhang et al. (2004)	Maize	Identification and hybridization	SS IIa	28%[a]	38%[a]	DP 6 to 11↑, DP 13 to 20↓
	Umemoto et al. (2004, 2008)	Rice	Identification	SS IIa		Similar to wild-type[a]	DP 7 to 11↑, DP 13 to 23↓
				SS I		Similar to wild-type[a]	Similar to wild-type
	Fujita et al. (2006)	Rice	Identification	SS I		Similar to wild-type	DP 6 to 7↑, DP 8 to 12↓, DP 16 to 19↑
	Ryoo et al. (2007)	Rice	Identification	SS IIIa	19%	23%	DP 6 to 8↓, DP 9 to 15↑, DP 16 to 20↓, DP 22 to 29↑
	Konik-Rose et al. (2007)	Wheat	Identification and hybridization	SS IIa	34%	44%	N/A
	Shimbata et al. (2012)	Wheat	Identification and hybridization	SS IIa	23%	31%	DP 6 to 10↑, DP 11 to 25↓, DP 25 to 35↑
Overexpression of GBSS	Flipse et al. (1996)	Potato	Development of a GBSS gene-dosage population	GBSS		No linear correlation between GBSS activity and amylose content	N/A
	Itoh et al. (2003)	Rice	Transformation	GBSS	20%	>40%	N/A
	Sestili et al. (2012)	Wheat	Transformation	GBSS		Similar to wild-type	N/A

↑: Proportion of chain lengths increases; ↓: Proportion of chain lengths decreases.
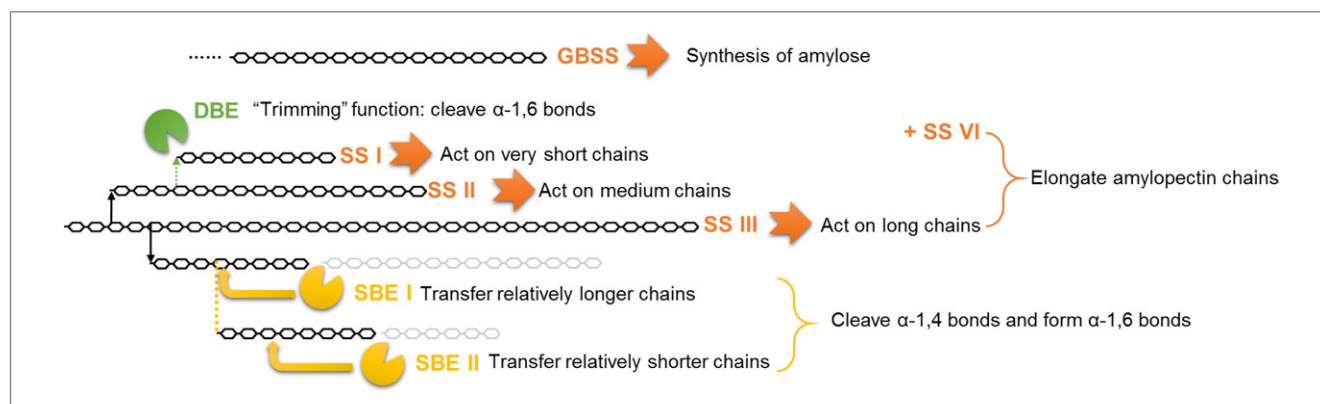[a] Determined by SEC/GPC.

Figure 2–Primary biosynthetic enzymes and their functional roles on starch molecular structure as described in Nakamura (2018) and Tetlow (2011). GBSS: granular-bound starch synthase, SS: starch synthase, SBE: starch branching enzyme, DBE: starch debranching enzyme.

amylopectin branches in HAS crystallize relatively rapidly into the more readily formed B-form, whereas shorter branches in normal starches crystallize more slowly into the less readily formed but more thermodynamically stable A-form (Gidley, 1987).

The lipids in cereal starches, mainly lysophospholipids and free fatty acids (Morrison, 1988), can be in free form or as part of amylose–lipid complexes. The complexes can be inferred from the V-type diffraction pattern (major peak at approximately 20° 2θ). Amylose and lipid contents are well correlated in waxy and nonwaxy cereal starches (for example, barley starch [Morrison, Tester, Snape, Law, & Gidley, 1993]), and lipid content tends to increase in mutants with high-amylose content (Pérez, Baldwin, & Gallant, 2009). Long chains of amylose seem to have higher affinity for lipids than short chains of amylopectin, while amylopectin chains already in double-helical forms cannot include lipids inside the helix. The amylose complex V-type fraction of total crystallinity increases with amylose content in barley HAS (Regina et al., 2012).

Glucan chains near branching points or long chains of amylopectin are not likely to form double helices, but make up the amorphous part of granules together with amylose (Wang et al., 1998). This disordered region contributes a featureless background in diffraction patterns. The known contribution from the amorphous part can be subtracted from the total area under the X-ray diffraction pattern to calculate the total crystallinity. Reduction of crystallinity along with alteration of A-type into B-type crystallinity in cereal HAS has been reported previously (Butardo et al., 2011; Huang et al., 2015; Regina et al., 2012; Yamamori, Fujita, Hayakawa, Matsuki, & Yasui, 2000).

Starch granules

The granular shape of HAS tends to be asymmetrical and deformed, for example, crescent-shaped in wheat and elongated in rice and maize, whereas normal starch granules have spherical or angular shapes. Native starch granules are birefringent with a Maltese cross pattern under polarized light, reflecting the radial organization of underlying molecular and crystalline structures. The cross pattern disappears to some extent in some granules of HAS (Slade et al., 2012), suggesting that HAS granules have a less ordered arrangement on the light wavelength scale of 100s of nanometers. Similarly, the characteristic 9- to 10-nm repeat structure in small–angle scattering patterns is weaker in HAS (Blazek et al., 2009; Regina et al., 2012), reflecting a less regular order at this shorter length scale. Normal wheat starch granules have

a bimodal size distribution: large lenticular granules (ca. 15 to 40 μm) and small spherical granules (ca. 1 to 10 μm). In mutant wheat starch with elevated amylose content, both large and small granules become crescent-shaped and shrunken (Regina et al., 2006; Slade et al., 2012; Yamamori et al., 2000). The percentage of elongated granules is up to 32% in high-amylose maize starch (Jiang, Campbell, Blanco, & Jane, 2010). A range of large voluminous and nonangular rounded or elongated and filamentous granular shapes is found in rice HAS (Wei, Qin, et al., 2010). Fused granule agglomerates are formed in the amyloplast, possibly due to enhanced amylose interaction on the outer layer of 2 starch granules (Jiang, Horner, et al., 2010).

Biosynthesis of High-Amylose Starch in Plants
Enzymes in relation to starch synthesis

Overview of the enzymatic machinery. Proceeding from the substrate ADP-glucose to the large glucan polymers in the amyloplast, the enzymatic machinery forms two types of glucan linkage: α-(1-4) and α-(1-6) glucosidic bonds, the latter forming branch points. The formation and cleavage of the bonds are mainly controlled by three types of enzymes (Figure 2): SSs, SBEs, and debranching enzymes (DBEs). SSs catalyze the elongation of glucan chains with ADP-glucose by forming an α-(1-4)-glucosidic linkage at the nonreducing end. SBEs cleave an internal α-(1-4)-linkage and transfer the cut chain to form an α-(1-6)-linkage at a different α-(1-4)-linked site. Multiple SBEs play a critical role in determining the branching pattern and the polymodal distribution of chain lengths (Bertoft, 2013; Hanashiro et al., 1996). DBEs (for example, isoamylase and pullulanase) have a "trimming" function through the cleavage of branches to allow the amylopectin molecule to crystallize properly (Myers, Morell, James, & Ball, 2000). Disproportionating enzymes (D-enzyme), which cleaves a glucan chain and transfers one portion to the nonreducing end of another preexisting chain, may have impacts on the generation of HAS as well (Tetlow, 2011; Tetlow, Morell, & Emes, 2004). SSs and SBEs have preferences to function on glucan chains with a certain DP range. These preferences have been deduced from the chain length distribution of amylopectin in mutant or transgenic lines lacking a specific enzyme, and from *in vitro* studies (Nakamura, 2018; Tetlow, 2011).

These synthesized enzymes may act on amylopectin coordinately by forming protein complexes. There is evidence suggesting that formation of complexes can alter the properties of enzymes. For example, even though only one biosynthetic enzyme isoform

is mutated, one or more other synthesized enzymes lose activity, as shown in maize (Colleoni, Myers, & James, 2003; Dinges, Colleoni, James, & Myers, 2003) and wheat (Tetlow, Wait, et al., 2004), or result in the dissociation of the complex (Tetlow, Wait, et al., 2004). However, the *in vivo* functional mechanism of the complexes is largely unknown (Zeeman, Kossmann, & Smith, 2010).

The relationships between the biosynthetic enzymes and amylose content have also been reviewed elsewhere (Bird & Regina, 2018; Nakamura, 2018; Regina et al., 2015; Tetlow, 2011; Tetlow & Emes, 2014; Wang, Hu, Chen, Liu, & Wei, 2017). To summarize, "apparent" amylose content, in principle, can be enhanced in three ways: (1) increase GBSS action to promote amylose synthesis, (2) decrease relative amylopectin levels through inhibition of SS(s), or (3) decrease amylopectin branching through inhibition of SBE(s). The reported studies have been summarized in Table 2, based on these three principles. Inhibition of SSs does not change amylopectin branching structure as much as inhibition of SBEs does, but it increases the proportion of linear chains (true amylose) by repressing amylopectin synthesis. In contrast, SBE inhibition results in more amylopectin being measured as "apparent" amylose content using iodine-based methods because of the longer branches. Perhaps, the proportion of linear chains in SBE mutants does not actually change as much as in SS mutants, but increasing amounts of amylopectin get classified as amylose as branching is inhibited (Jobling et al., 1999; Morell et al., 2003). The subsections will discuss the functions of SSs and SBEs on the lowest level structure, individual branches, which determines many higher level properties. Accurate manipulation of starch molecular structure is of interest, which allows fine-tuning the design of starch with desirable properties.

Starch branching enzyme. Mutants or transgenic plants lacking SBEs show significantly elevated apparent amylose contents. There are two major types of SBE (SBEI and SBEII). In the tubers of potato, SBE I (also called class B) is the major isoform, while SBE II (also called class A) is expressed at very low level (Jobling et al., 1999). However, in cereals, SBE II significantly affects amylose content, whereas there is no or only little change in phenotype for SBE I mutants of cereal grains (Blauth et al., 2002; Regina et al., 2004; Satoh, Nishi, Yamashita, & Takemoto, 2003). SBEII is further divided into two isoforms (IIa and IIb), which have high similarity in sequence and molecular weight. For example, the mutants of *amylose-extender* (*ae*) gene, which is related to SBE IIb in maize and rice, show a rise in amylose content, along with longer branch chains in amylopectin (Jane et al., 1999; Nishi, Nakamura, Tanaka, & Satoh, 2001). SBE IIa is the major isoform in the soluble phase of the wheat and barley endosperms. It is suggested to be more important than SBE IIb in controlling glucan branching and amylose content in wheat or barley, since the starch structure of transgenic wheat in which SBE IIb is silenced alone shows no significant change (Regina et al., 2006, 2010). Further, mutants deficient in both SBEIIa and SBEIIb have a higher level of apparent amylose content than suppression of either alone (Regina et al., 2010; Zhu et al., 2012). Starch branching enzymes (SBE I, SBE IIa, SBE IIb) in barley simultaneously silenced by hairpin-RNA generated "amylose only" HAS (Carciofi et al., 2012). A few studies also reported elevated amylose content in crops through downregulation of SBE as shown in Table 2.

There are two approaches to understanding the chain-length transfer range preference of SBEs: (1) *in vitro* study of purified SBE isoforms on glucose polymers and (2) deduction from the analysis of starch fine structure of mutants or transgenic lines lacking SBE

isoforms (*in planta* evidence). *In planta* functions of SBE isoforms on amylopectin branches are substrate-dependent (Tetlow, Morell, et al., 2004). SBEII isoforms prefer to act on amylopectin and form shorter branches (DP 6 to 14), whereas SBEI prefers amylose as a substrate and forms relatively longer chains (up to DP 30, but predominantly DP 10 to 13) (Tetlow & Emes, 2014). With regard to the difference between SBEII isoforms, it has been proposed that SBE IIa forms chains with DP 6 to 15, whereas SBE IIb forms short chains with DP 6 and 7 (Nakamura, 2018; Nakamura et al., 2010). Compared to SBE IIa, SBE IIb may have a broader affinity to amylopectin branches, allowing the wide range of branches to be shortened into a relatively narrow range.

Amylose, compared to amylopectin, has a small number of branches, suggesting that SBEs are also active in the synthesis. The role of SBEs in determining amylose structure should therefore not be ignored, even though there is lack of *in planta* evidence. The *in vitro* reactions of SBEs on linear "amylose" (Nakamura et al., 2010) have shown that SBE IIa and IIb from rice endosperm have no activity on short "amylose" (about 50 DP), but react on longer "amylose" (about 6000 DP). In addition, SBE I appears to have relatively broad affinity to amylose, not only showing similar change patterns as SBE II on long "amylose," but also predominantly transferring chains with about 30 DP from short "amylose."

Starch synthase. There are five classes of SSs based on their conserved primary amino acid sequences. One class is known as granule-bound SS (GBSS), which, as the name suggests, is only found bound tightly within starch granules. The other 4 SS classes (SSI, SSII, SSIII, and SSIV), known as soluble SSs, are either soluble in the amyloplast stroma, or partially soluble and partly associated with the granule (Zeeman et al., 2010). The soluble SSs are primarily involved in amylopectin synthesis, while GBSS is essential for amylose synthesis. Some soluble SS isoforms have distinct roles in amylopectin elongation. SSI, SSII, and SSIII classes seem to preferentially elongate short, medium, and long chains to a critical chain length, respectively (Tomlinson & Denyer, 2003). Fujita et al. (2006) suggested that SSI generates short-chain DP 8 to 12 from DP 6 to 7 chains in rice. The intermediate chains of DP 11 to 25 were elongated from DP 6 to 10 chains by SSII in wheat (Yamamori et al., 2000). Relatively longer chains (DP ≥ 30) were decreased due to SSIII deficiency in a rice mutant (Ryoo et al., 2007). The deficiency in SSs appears to be less effective to increase apparent amylose content compared to SBEs (Table 2).

GBSS deficiency results in waxy starch with greatly reduced amylose content, and may be involved in the elongation of long chains of amylopectin (Yoo & Jane, 2002). However, attempts to increase the expression of GBSS have not significantly increased amylose content (Flipse, Keetels, Jacobsen, & Visser, 1996; Sestili et al., 2012). The dosage of GBSS protein might not be a key factor to increase amylose content, if the synthesis is instead limited by other factors, including the availability of physical space within the matrix of amylopectin, and the availability of ADP-glucose and malto-oligosaccharides (Sestili et al., 2012).

Genetic approaches to elevate amylose content

Identifying mutant genes of SSs and SBEs. Strategies to elevate amylose content are mainly focused on suppressing the expression of SBEs or SSs, or elevating the expression of GBSS in the amyloplast (Table 2). The candidate genes coding for the enzymes are traditionally identified through mutational analysis that detects the deactivated gene as a newly presented phenotype. For example, in the *sex6* mutant of barley, sequence analysis showed that an early stop codon, which is caused by a G to A transition

on chromosome 7H, suppresses C-terminal translation of the active site of SSIIa (Morell et al., 2003).

In addition to natural mutations, mutants can be created physically (for example, by gamma ray and heavy ions) or chemically (for example by ethylmethane sulfonate that produces primarily C/G to T/A transitions). For either natural or induced mutagenesis, a key step is to identify the knocked-out genes, typically caused by single-nucleotide polymorphisms (SNPs). After identification of allele mutations, crossing plant lines with null alleles is used to generate homozygous null genotypes. Targeted induced local lesions in the genome (TILLING) was employed in the development of wheat HAS (Slade et al., 2012): high-amylose contents (up to 56%) were generated by cross-breeding the wheat lines with the new mutant alleles in SBE II. This method allows identification of mutants without the need for direct selection of phenotypes. This advantage is extremely useful for screening mutated alleles in polyploid genomes, for example, hexaploid genome of wheat (*Triticum aestivum* L.) that is composed of three independently maintained but closely related genomes, A, B, D. Unlike diploids, such as maize and barley, single-genome mutation in wheat thus usually does not result in any major changes in phenotype. Whether SNPs happen in the region of interest (ROI) of genomes can be instead visualized by high-resolution denaturing high-performance liquid chromatography (DHPLC) (McCallum, Comai, Greene, & Henikoff, 2000). However, recent sequencing technologies enable the more accurate detection of SNPs relevant to starch structures, for example, genotyping-by-sequencing (Butardo et al., 2017). TILLING in combination with chemical mutagenesis does not rely on transgenic technologies that are not always broadly acceptable to consumers (Slade, Fuerstenberg, Loeffler, Steine, & Facciotti, 2005). Regina et al. (2015) proposed that a screening procedure using an affinity gel electrophoresis system to first purify SBE II isoforms, followed by immunoblotting with anti-SBEIIa serum and sequencing, identifies SNPs of SBE IIa and IIb. The wheat lines after repeatedly crossing of null genotypes screened by this method have approximately 85% amylose content (iodine colorimetric value) (Regina et al., 2015). Another method to access and identify genetic diversity is multiparent-advanced generation intercross (MAGIC). MAGIC is also a powerful high-throughput screening approach for polyploid crops (Cavanagh, Morell, Mackay, & Powell, 2008) and diploid crops (for example, rice [Bandillo et al., 2013]). Overall, the identification of SNPs, together with effective mutagenesis, expands resources for phenotypic variants with desirable alleles and breaks the limitation of the relatively small germplasm pool accessible to plant breeders (McCallum et al., 2000; Slade et al., 2005, 2012).

Downregulation of SBE by RNA interference (RNAi). Gene modifications through RNAi have been used to generate high-amylose-content starches in potato, wheat, barley, and rice. The RNAi construct, including hairpin RNA, is designed by cloning cDNA fragments of the biosynthetic enzymes. The construct is expressed in the transgenic plants to suppress the transcription of targeted genes. The method offers an alternative way to downregulate SBE to generate HAS. Especially for polyploid crops, such as wheat, it is challenging to screen mutated alleles and combine them to have homozygous mutations on homologs that have new phenotypes. Regina et al. (2006) designed hairpin–RNA construct targeting SBEIIa and SBEIIb using the exon 1 to 3 region in inverted repeats that are separated by the intron 3 of the respective genes. In addition, SBE was specifically targeted for silencing in potato (Jobling et al., 1999; Schwall et al., 2000) and rice (Butardo et al., 2011) to successfully generate HAS.

High-Amylose Starch and Resistant Starch Formation

Amylose content is found from many (mostly *in vitro*) studies to be correlated with RS content. Attempts to estimate RS content *in vitro* initially focused on a simple time-based classification. Specifically, the undigested starch remaining after *in vitro* hydrolysis by excessive pancreatic amylase and amyloglucosidase at 37 °C for 120 min was believed to enter the large intestine physiologically (Englyst, Kingman, & Cummings, 1992). However, the *in vitro* hydrolysis conditions vary among several analytical methods to determine RS content. Although *in vitro* prediction is validated with limited *in vivo* data derived from digesta collected from human ileostomy subjects (Englyst, Kingman, Hudson, & Cummings, 1996), arguments are put forward on limitations of *in vitro* data, most importantly the complex physiological processes and individual differences in gastrointestinal passage rates and enzyme secretion. Further, the term "RS" may be misleading to suggest that the fraction is definitely indigestible or resistant to amylases, despite what its name suggests (Dhital et al., 2017). There is evidence showing no biochemical difference between undigested residues and native granules (Cai & Shi, 2013; Evans & Thompson, 2004) and recovery of the *in vitro* digestion pattern by redigestion of the "resistant" fraction (Zhang, Ao, & Hamaker, 2006). On the other hand, "resistance" may be derived from the depletion of the available substrate or enzymatic activity loss due to hydrolysis product through binding to amylase. Therefore, given sufficient time and optimal hydrolysis conditions, there is no undigestible starch containing physiologically cleavable glycosidic bonds, α-(1-4) or α-(1-6), by brush border enzymes (Dhital et al., 2017). In fact, the fraction of starch entering the colon should be dependent on both physiological digestion rate and gastrointestinal passage rates, which are highly simplified in the *in vitro* digestion, even in a standardized method (Minekus et al., 2014). However, *in vitro* assays have been widely used, because of the advantages in quick evaluation of RS level (for example, screening of newly derived HAS cultivars) and relatively low cost. Rather than only comparison of experimental data from HAS studies, focus on the fundamental but rate-determining steps of the conversion of HAS into absorbable products allows a better understanding of the effect of elevation of amylose content on digestibility. The two fundamental steps are the diffusion or absorption of the enzyme onto the substrate and the catalytic event. The catalytic event happens once digestive enzymes bind to starch substrate, either in native granular form and the forms induced by food processing. However, the structural features in the native granular form of starch, as 1 end of the spectrum of starch types in foods, should not be neglected, especially for HAS that have more heat-stable granule structures.

Access to starch substrate

Barriers, including plant cell wall and protein matrices, prevent or slow down enzyme diffusion to substrate and thus alter the digestion rate. Previous studies have reported a reduction of glycemic responses (C. H. Edwards et al., 2015) and excretion of undigested macronutrients (Ellis et al., 2004; Noah et al., 1998; Tovar, Bjorck, & Asp, 1992) on consumption of whole grains or cooked legumes in humans and rats. The *in vitro* digestion rate of starch and lipids was reduced due to the intact cell walls (Dhital, Bhattarai, Gorham, & Gidley, 2016; C. H. Edwards, Warren, Milligan, Butterworth, & Ellis, 2014), whose roles in macronutrients encapsulation have been reviewed recently (Grassby, Edwards, Grundy, & Ellis, 2013; Grundy et al., 2016). The intactness of cell walls is related to particle size, which is dependent on mastication habits and processing conditions, for example, milling and heating. Given the diameter

of individual grain cells can be less than the food particle size, the larger the particle size, the less disrupted the cell walls after milling. The particle size of whole grains is negatively correlated with amylase digestibility, as shown in milled barley and sorghum grains (Al-Rabadi, Gilbert, & Gidley, 2009). After heating, the intact cell wall remains as a limiting factor of the hydrolysis of the encapsulated starch, as examined using thermally isolated intact cells at 95 °C (Dhital et al., 2016). As for the particle size of starch granules, smaller granules normally have more available surface area for the adsorption of enzymes, with the exception of granules containing a surface-accessible interior surface area (cavities or radial channels) (Dhital, Shrestha, & Gidley, 2010). Compared with larger counterparts, smaller granules have higher amylolysis rates, as demonstrated by the digestion of size-fractionated native starches (Dhital et al., 2017). In SBE-deficient transgenic rice, starch granules that consist of smaller subgranules have increased granule diameters but seem to be fragile to mechanical processing due to the hollow interior (Wei, Xu, et al., 2010). However, this reduced surface area of enzyme adsorption may or may not reduce the hydrolysis rate and extent of the HAS. The accessibility of enzymes to substrate also depends on the damage to starch granules during processing, for example, milling, which may cause a higher degree of damage to fragile granules, and exposure of the starch granule surface that may be covered by protein matrices. The effect of bound protein matrices on HAS digestion has not been reported. Soluble fibers (nonstarch polysaccharides) also generate such barriers. Arabinoxylans and β-glucan are the primary soluble fibers in cereals. These components affect viscosity of digesta, which potentially controls the diffusion rate of enzymes, but the viscosity effect can be negated by intense mixing (Dhital, Dolan, Stokes, & Gidley, 2014). Thus, other possible mechanisms, such as interaction of enzymes and fibers, and fibers coating the starch granules, need further investigation.

Structural features of HAS slowdown hydrolysis

Structural features of native HAS and enzymatic resistance. At the supramolecular level (μm), the starch granules with pores (>0.1 μm) have an "inside-out" digestion pattern allowing direct access for enzymes to diffuse inside the granules, whereas those without pores show surface erosion or pitting at the early stage of digestion and thus limited accessible surface area for enzyme binding (Dhital et al., 2010). For native starch with regular amylose content, surface pores and radial channels can be observed by scanning electron microscopy (SEM) in starch with A-type crystallinity, that is, cereal starch (maize, wheat, barley, and rice). However, pores on the granules are not uniform. Some granules contain many pores, others a few, and some none (Buléon, Colonna, Planchot, & Ball, 1998). The internal surface area increases the accessible binding area for amylases. But starch with B-type crystallinity normally does not have pores, for example, potato starch and high-amylose maize, which may have amylose and amylopectin tightly interacting at the periphery that is not permeable to amylase (Dhital et al., 2017). Surface pores were reported in SBEIIb-deficient rice lines (Dhital, Butardo, Jobling, & Gidley, 2015), whereas no surface pores were found in an SBEIIb- and SBE I-deficient rice line (Wei, Xu, et al., 2010).

After adsorption of the enzyme onto the substrate, the glucan chains must properly fit into the active sites of enzymes before hydrolysis of glucosidic bonds. Catalysis on normal starch and HAS can be compared in terms of semicrystalline organization (A-type crystallinity compared with B-type crystallinity) and chain length of α-glucan (short chain compared with long chain).

At the submolecular level (<10 nm), the double-helical structure does not fit into the active site of α-amylases. After heating above the gelatinization onset temperature, helical structures start melting and become soluble, provided that there is sufficient water. Gelatinized starch, containing more expanded amylopectin, is much more susceptible to enzymes than native starch, in which amylopectin is packed in an orderly semicrystalline form.

Native cereal starches normally show an A-type crystalline pattern, whereas those with amylopectin of longer chain length than their normal starch counterparts give the B-type crystalline pattern. The digestibility of A- and B-type crystallinity has been compared to model systems in which the same starch sources were used to generate different types of crystals by varying preparation methods. One study using amylose spherocrystals (DP 15 to 20) (Planchot, Colonna, & Buleon, 1997; Williamson et al., 1992) suggested that the B-type is less susceptible to enzymes than the A-type, while one using debranched waxy starches (Cai & Shi, 2010, 2013; Cai, Shi, Rong, & Hsiao, 2010) showed no difference between A-type and B-type. However, studies using model compounds to generate crystals may not be representative of the lamellar structure derived through biosynthesis in amyloplasts (Dhital et al., 2017). The digestibility of both polymorphs may be not only determined by the organization of double helices: different levels of granule organization may also jointly affect the digestibility of native starch. On the one hand, double helices are more tightly packed in A-type crystals, as the monoclinic lattice is thermodynamically more stable and has a higher local molecular density, possibly leading to lower enzymatic susceptibility. On the other hand, the B-type hexagonal lattice is proposed to form larger "blocklets" (200 to 500 nm) than crystals in A-type starches (20 to 120 nm) at the periphery of starch granules, and thus there are no pores and channels to allow enzyme directly diffuse towards the hilum (Gallant, Bouchet, & Baldwin, 1997). There is no direct evidence showing that long chains are intrinsically more resistant to hydrolysis than short chains. However, long chains in HAS are hypothesized to stabilize the crystalline structures by extending through multiple crystals, which thus potentially contribute to the resistance in HAS (Jane et al., 1999; Zhang, Ao, & Hamaker, 2008).

RS formation in HAS during food processing. Based on the source of enzyme resistance, there are five types of RS. RS type 1 (RS1) is sourced in whole grains or seeds in which protein matrices and/or cell wall materials make the starch inaccessible to enzymes. RS type 2 (RS2) is attributed to raw starch granule structures, that is, helical and crystalline structure that is less susceptible to enzymes. However, the crystalline structure can be melted (gelatinized) during the heat treatment that is widely employed in food processing. Reassociation of starch chains (retrogradation) after melting forms RS, termed RS type 3 (RS3). RS type 4 (RS4) is generated by chemical modifications. Recently, amylose–lipid complexes were proposed as RS type 5 (RS5). The elevation of amylose content improves the potential sources of RS2, RS3, and RS5 that are dense molecular structures. The concept of local molecular density (Zhang, Dhital, & Gidley, 2015) underlies enzyme resistance mechanisms of these three types of RS, since the molecular and crystalline structure plays a fundamental role in the formation of the three types of RS (Figure 3).

Retention of helical structure (RS2). HAS is more thermally stable than the corresponding native starch. This allows HAS a higher possibility to retain a semicrystalline structure under conventional cooking conditions, whereas gelatinized starch is highly susceptible to enzymatic hydrolysis (Tester, Qi, & Karkalas, 2006). The loss of
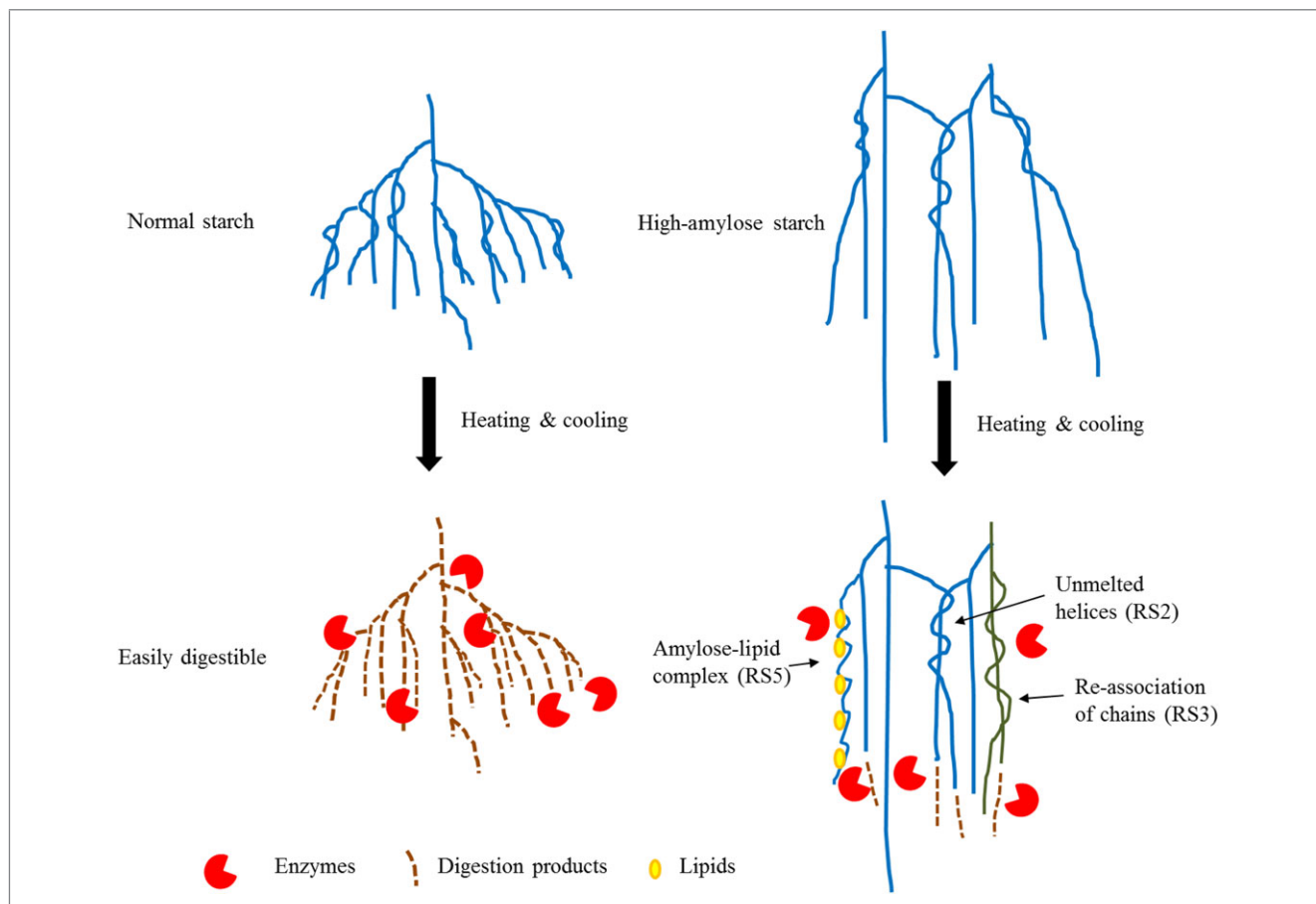
Figure 3–Sketch showing possible mechanisms of formation of resistant starch (types 2, 3, and 5) in high-amylose starch. Increasing the amylose content will increase the thermal stability of the native starch granules that are more likely to retain crystallinity (RS2) during processing such as cooking, baking, and frying. Even if the crystalline structure is destroyed during cooking, the higher percentage of amylose tends to retrograde (RS3) faster and to a larger extent. More amylose or longer branch chains can complex with lipids (RS5) during heating and cooling. Together, increasing the amylose content results in more dietary resistant starch.

semicrystalline structure during heating is irreversible. The thermal motion of glucan chains and water dissociates double helices, along with birefringence loss as observed under polarized light and endothermic peaks found in differential scanning calorimetry (DSC) thermograms. Previous studies have shown that HAS starts to gelatinize at a higher temperature than the corresponding native starch (Jane et al., 1999; Regina et al., 2012), possibly due to the existence of longer double helices that have a higher melting temperature. On the other hand, some of the amorphous regions may be mobilized at the beginning of gelatinization, since these regions are more likely to be easily accessible to free water. However, some of the amorphous region contains double helices as suggested by the observation that double helix content is higher than crystallinity (Cooke & Gidley, 1992). Double helices of HAS extending into the amorphous region possibly stabilize the amorphous region, which consequently increases the initial melting temperature.

A reduction of glycemic and insulinemic indices was attributed to the addition of high-amylose maize starch into white wheat flour with a ratio of 2:3 (Hoebler, Karinthi, Chiron, Champ, & Barry, 1999). The high-amylose maize incorporated into bread was incompletely gelatinized and retained B–type crystallinity after bread processing at a baking temperature of 250 °C. The retained crystallinity at that temperature also contributes to the limited water content in bread. Water content in food materials determines

the extent and temperature range of gelatinization (Liu et al., 2009). Starch granules tend to retain native characteristics in foods with low-moisture content during processing, for example, the surface regions of breads or biscuits, which lose water quickly during baking (Zhang & Datta, 2006).

Reassociation of chains (RS3). The irreversible process of starch gelatinization in water includes water uptake, swelling, double helix melting, crystallite loss, starch solubilization, and finally, reassociation (retrogradation) during cooling. Amylose and amylopectin have different retrogradation properties. The retrogradation process is generally associated with a viscosity increase (also termed setback). Amylose acts as a cross-linking agent to increase intermolecular associations and the continuity and firmness of the gel network, while waxy starches without amylose normally show rapid loss of viscosity on shearing after formation of pastes. Amylose is preferentially leached out from granules as a random coil in a freshly prepared aqueous solution. The random coil tends to form either single-helical complexes with suitable complexing agents (such as lipids to form lipid–amylose complexes) or double helices by self-association (Jane, 2009; Jane & Robyt, 1984; Leloup, Colonna, Ring, Roberts, & Wells, 1992). Although a branch point of amylopectin could interrupt the interchain association (Miles, Morris, Orford, & Ring, 1985), three types of molecular interaction leading to retrogradation can be envisaged in HAS, including (1) amylose–amylose,

(2) amylose–amylopectin, and (3) amylopectin–amylopectin. The rate of the amylose self-interactions depends on amylose chain-lengths, concentration and cooling rate, with the maximum rate found for approximately 100 DP amylose (Gidley & Bulpin, 1989). The minimum requirement of chain length to form double helix in a pure oligosaccharide solution is 10 DP (Gidley & Bulpin, 1987), while the chain-length distribution of amylopectin branches peaks at around 12 (Hanashiro et al., 1996). Thus, the more linear structure of amylose or intermediate amylopectin in HAS with less steric blockage of stretched branched chains leads to a higher tendency to form a stable gel. Retrograded amylose with a double-helical structure is suggested to be resistant to amylolytic hydrolysis (Jane, 2009; Jane & Robyt, 1984).

Formation of amylose–lipid complex (RS5). All intragranular lipids naturally complex with amylose in raw starch (Morrison et al., 1993), while free lipids (nonstarch lipids) exist and could complex with amylose during gelatinization (Morrison, 1988). Other complexing agents, including iodine, alcohols, and fatty acids, also facilitate the formation of single-helical complexes. Cereal starches normally contain about 1% fat, while tubers, legumes, and waxy cereal starches are virtually fat-free (Becker, Hill, & Mitchell, 2001). Lipid content tends to increase in mutants with high-amylose content (Pérez et al., 2009). Thus, HAS may be expected to contain more natural amylose–lipid complexes. The complex normally melts at a higher temperature than that at which amylopectin double helices dissociate, as judged by DSC thermograms. The thermal stability of the complex could thus cause it to retain its structure after thermal processing. Further, the complex is reformed during cooling, a process that is relatively fast compared to reassociation of double helices in RS3. Given more lipid content as a complexing agent in HAS, the heat treatment of food processing enhances the possibility of complex restoration or new complex formation. On the other hand, if lacking the complexing agents, amylose tends to remain as a random coil or to form double helices as a lower energy and as a stable form (Jane, 2009). The long chains of amylopectin in maize HAS have shown similar efficiency to amylose in complexing with fatty acids (Hasjim, Ai, & Jane, 2013). The complex can be either amorphous or crystalline in form. The crystalline form displays V-type peaks in X-ray diffraction. Heat treatment at a temperature above the melting temperature of the amorphous complex (typically 94 to 100 °C in excess moisture) and below that of the crystalline complex (typically 100 to 125 °C in excess moisture) converts an amorphous complex into a crystalline complex (Jane, 2009). An amylose–lipid complex reduced the *in vitro* digestibility of starch (Ai, Hasjim, & Jane, 2013; Cui & Oates, 1999) and postprandial glycemic and insulinemic responses in humans and rats fed with HAS of maize prepared to enhance the content of amylose–lipid complexes (Hasjim et al., 2010; Zhao et al., 2011).

High-Amylose Starch in Food and Potential Health Benefits
Health claims for HAS

HAS, identified as RS type 2, generally satisfies the definition of dietary fiber by food regulatory agencies. However, the Inst. of Medicine (IOM, US Natl. Academy of Sciences) regards RS that is naturally occurring and inherent in a food or created during normal processing of a food as "dietary fiber," while RS obtained through any isolation or extraction process (for example, chemical, enzymatic, or aqueous steps) should be categorized as "functional fiber" (IOM, 2005). The U.S. FDA has proposed a similar defi-

nition of "dietary fiber" that divided food ingredients into those that are naturally occurring and those that are isolated or synthetic in the Nutrition and Supplement Facts label final rule in 2016. Declaring food ingredients in the latter group as "dietary fiber" in nutrition labeling will require FDA approval after assessments of scientific evidence relating the components to physiological benefits of human health. Under the proposed scheme, HAS (RS2) in the form of whole grain remains in the "dietary fiber" group, unlike other types of "synthetic" RS (retrograded (RS3), chemically modified (RS4), and synthesized with lipids (RS5)).

Current regulations identify potential physiological benefits of RS. The European Food Safety Authority (EFSA) has approved an RS-relevant health claim that "Replacing digestible starch with RS induces a lower blood glucose rise after a meal" (EFSA, 2011). Food Standards Australia New Zealand (FSANZ) considered RS as a type of dietary fiber and noted that scientific studies demonstrated that RS promotes modulation of blood glucose through reducing peak postprandial blood glucose concentration and promotes laxation. Manufacturers in the United States are allowed to use terms such as "resistant" or "indigestible" in food labeling with the corresponding starch names legally approved and clearly labeled (Nugent, 2005). FDA permits a qualified health claim that "High-amylose maize resistant starch, a type of fiber, may reduce the risk of type 2 diabetes, although FDA has concluded that there is limited scientific evidence for this claim" in 2016. For calorie labeling, RS has a lower energy value (2 kcal/g) compared to carbohydrates (4 kcal/g) in Europe (Lockyer & Nugent, 2017), as well as in Australia and Japan (Nugent, 2005). In the United States, RS is assigned to insoluble dietary fiber that has 0 kcal/g.

A widely used analysis method for RS is AOAC Official Method 2002.02 for HAS labeling purposes. For example, as assessed by this method, uncooked maize HAS (HYLON VII, 70% amylose content) contains 50% RS, whereas the value is 0.5% in regular maize starch (McCleary, 2007). However, the Englyst assay is a well-accepted method to measure RS content in food materials for dietary intervention studies. The specific methods for RS stimulate physiological conditions through validation with *in vivo* RS results from ileostomy patients. It should be noted that RS values quantified by the specific methods should not be summed to give a value of 'total' dietary fiber, as measured by AOAC 985.29 or 991.43, to avoid double-counting. The simple summation may potentially overestimate RS content, since the "total" dietary fiber methods measure some of the RS (McCleary, 2013).

Applications of HAS

HAS provides a wide range of new features in nutrition, food processing, medication, and industrial use, compared to normal starch (Figure 4). Starch-based food plays a predominant role to fulfill the calorific requirement of human diets. HASs as RS ingredients can be added into food products for high fiber and low-calorie labeling claims.

To supply ingredients for large-scale food production through extensive cultivation of crops with elevated amylose content raises an important question about the starch yield of the modified crops. The mutant or transgenic lines with high-amylose content were generally reported to have a lower grain yield than the wild type (Butardo et al., 2011; Hazard et al., 2015; Schonhofen, Hazard, Zhang, & Dubcovsky, 2016; Schwall et al., 2000). Schwall et al. (2000) reported that the starch yield of an SBE-deficient potato line was halved. Durum wheat lines with a mutation in SBE II enzyme had a 15% reduction in grain yield compared to the wild type (Hazard et al., 2015), while a similar level of reduction
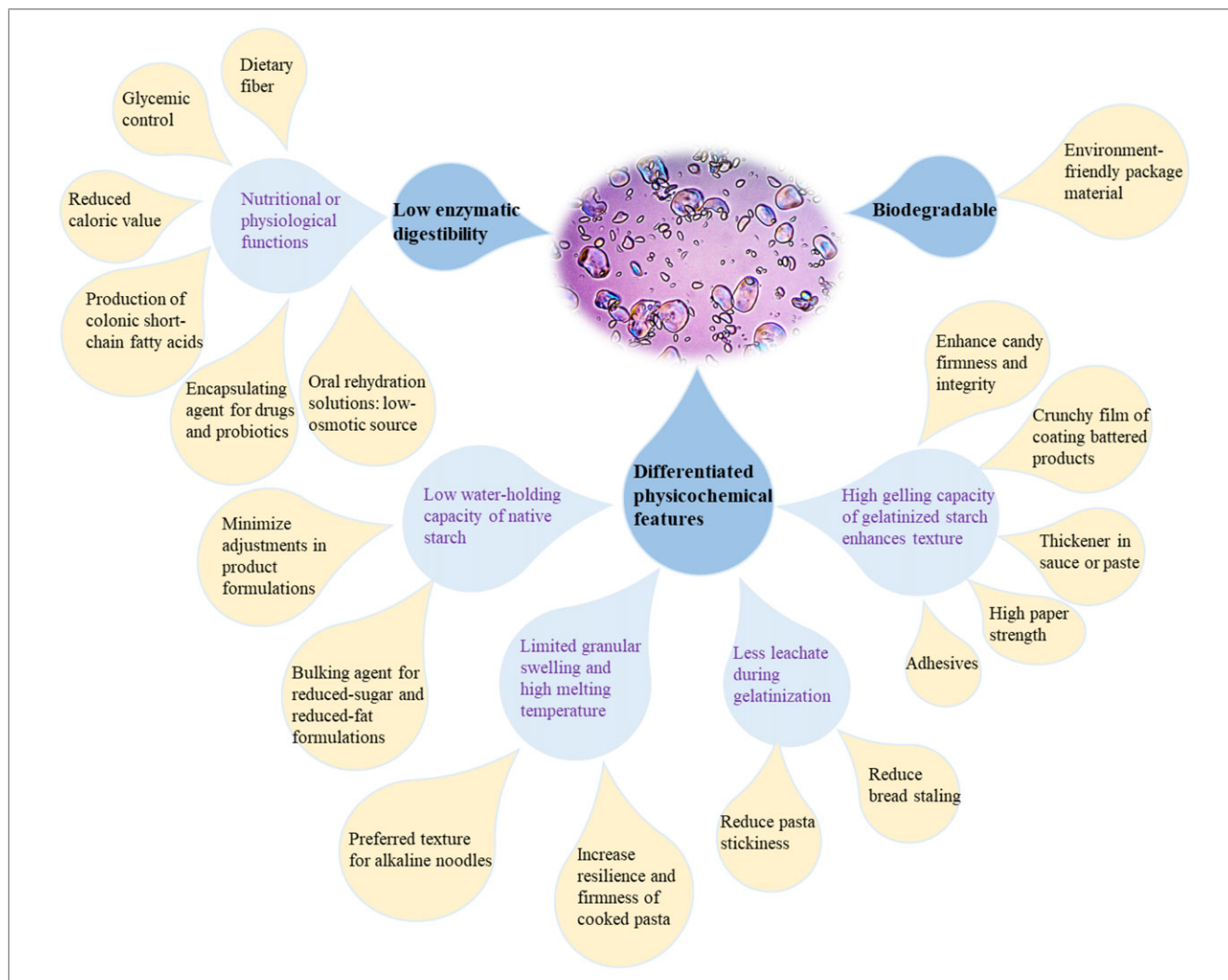
Figure 4–A diagrammatic illustration of high-amylose starch applications. The central picture is high-amylose wheat starch under polarized light.

was reported in SBE II mutant wheat but the reduction was not significant (Schonhofen et al., 2016). The downregulation of SBE in wheat seems to have a lower yield penalty.

Even though HAS retains the traditional properties of starch (white in color, fine particle size, and neutral flavor), the thermal and pasting behaviors usually show significant changes that, in turn, alter the food sensory characteristics. Increasing apparent amylose content is generally associated with higher gelatinization temperature and increased rate of retrogradation. The high gelatinization temperature could either be an advantage to obtain desirable qualities in starch-based foods or a disadvantage to cause processing difficulties (for example, cooking time and temperature of food products) (Figure 5). It is possible that the digestion resistance becomes similar above a minimum amylose content. For example, high-amylose maize starch with an apparent amylose content at 50% or 80% level shows little differences in terms of *in vitro* enzyme digestibility (Shrestha et al., 2015). In the cases that a lower gelatinization temperature is preferred, the intermediate level of apparent amylose starches may be more appropriate without needing to compromise for RS/fiber content.

Dough-making is an essential intermediate step to transform flours into products such as bread, pasta, noodle, and so on. The dough properties influence final sensory features of the food prod-

uct. The dough prepared with HAS wheat flour has distinct properties (Morita et al., 2002; Van Hung, Maeda, & Morita, 2006). While the dough contains starch of the native form, the effect of amylose content on the dough properties is ambiguous (Van Hung et al., 2006). As the filler in the gluten protein network, HAS granules with distinct granular surface and size distribution may affect dough rheological properties (Larsson & Eliasson, 1997). The other components, such as proteins and lipids, have significant effects on dough properties (Goesaert et al., 2005; Graybosch, Peterson, Moore, Stearns, & Grant, 1993). Compared to regular wheat flour, the high-amylose wheat flour containing higher amounts of protein and lipids produced a harder and more viscous dough (Morita et al., 2002; Van Hung, Yamamori, & Morita, 2005). These reports are for wheat flour with no more than 38% amylose. Now that HA wheat flour with much higher amylose content is available (Regina et al., 2015; Slade et al., 2012), it will be interesting to compare the effect of different elevated levels of amylose on wheat dough and food properties. The dough elasticity and viscosity largely depend on the gluten quality. However, the gluten components in HAS wheat has rarely been investigated.

After heat processing of wheat dough, the leached amylose and amylopectin act as cross-linking agents to hold swollen starch
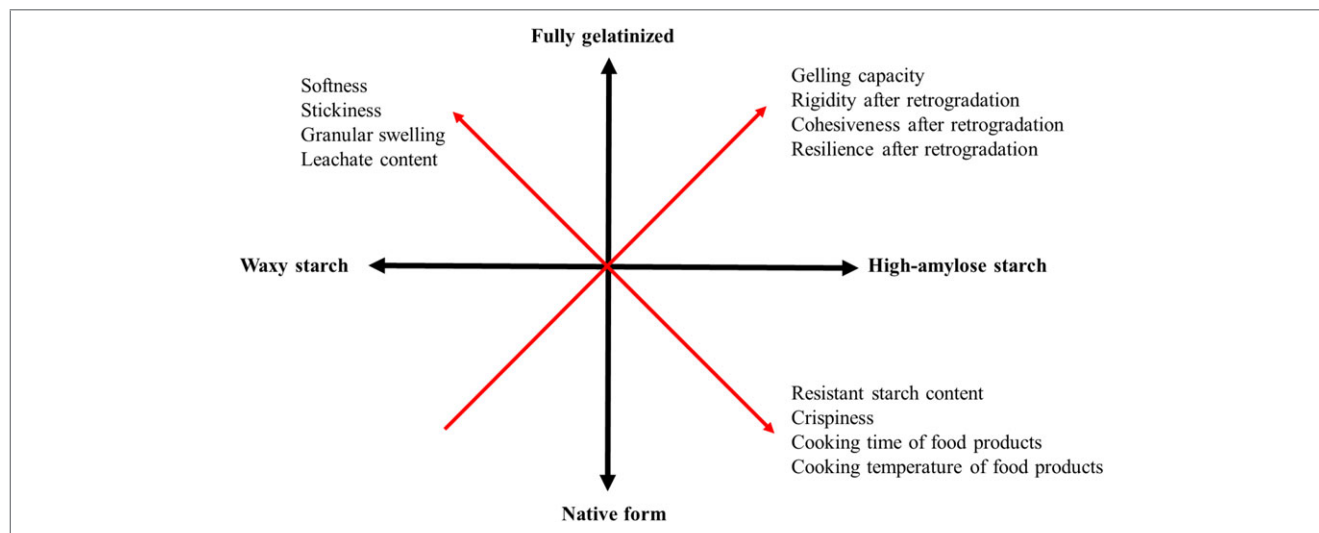
Figure 5–Two-dimensional plot illustrating the synergistic effect of amylose content and gelatinization extent on eating qualities of starch-based food. The eating qualities are enhanced along the arrow direction. Left-up arrow: a combination of low-amylose content and high extent of starch gelatinization will tend to have elevated softness and stickiness due to increased granular swelling and leachate content. Right-up arrow: a combination of high-amylose content and high extent of starch gelatinization will tend to enhance gelling capacity and properties (rigidity, cohesiveness, and resilience) of food containing retrograded starch. Right-down arrow: food products containing high-amylose starch with limited gelatinization require more cooking time and higher cooking temperature, while retaining the native form of starch leads to higher resistant starch content and crispiness.

granules and gluten together (Li, Dhital, & Wei, 2017). As discussed above, due to higher gelatinization temperature than the corresponding normal starch, the dispersion of amylose and amylopectin in HAS into an aqueous solution is hindered in the limited swollen granules. The limited swollen granules may negatively affect the expansion of dough during baking, with granules remaining trapped in the gluten matrix, preventing the loaf from expanding greatly. Bread-staling during storage is enhanced by recrystallization of intergranular amylose and amylopectin. The incorporation of HAS with generally less leachate may help to reduce bread-staling.

The amylose content also affects pasta quality. Amylose in pasta contributes to resilience and firmness that primarily determine the cooking quality of pasta. Soh, Sissons, and Turner (2006) improved spaghetti cooking quality with a significant increase in pasta firmness and a reduction in pasta water uptake by combining durum wheat flour with high-amylose maize starch (ranging from 27% to 74%). The optimum level of amylose was suggested to be 32% to 44%. The textural attributes of the pasta with added HAS showed no significant changes in terms of pasta cooking loss, texture, and sensory properties (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2013). The pasta made from high-amylose semolina from SBEII mutants shows a favorable increase in firmness but undesirable color and cooking properties (Hazard et al., 2015).

The relationships between ratio of amylose/amylopectin and noodle-eating qualities have been reviewed elsewhere (Li et al., 2017). The desirable sensory features vary with noodle type, with white salted noodles (WSNs) and yellow alkaline noodles (YANs) as the two most widely consumed noodles. The optimal level of amylose content in WSN was suggested to be 21% to 24% and highly swelling starch (for example, waxy starch) is preferred (Guo, Jackson, Graybosch, & Parkhurst, 2003). Reconstitution with HAS and waxy starch in WSN might be a solution to obtain high-fiber noodles without losing desirable qualities. Compared to WSN, the texture of YAN tends to have a higher level of firmness and hardness of mouthfeel, which can be potentially enhanced

by increasing amylose content (Baik & Lee, 2003; Guo et al., 2003).

HAS and health benefits

Human consumption studies (Figure 6) show that the digestibility of HAS is generally lower than normal starch, which is fundamental to any proposed mechanisms of health benefits. The risk of type 2 diabetes could be effectively reduced through reducing postprandial glucose responses and enhancing colonic fermentation. These two mechanisms are dependent on the digestion rate and extent of dietary carbohydrates. Beta cells in the pancreas of type 2 diabetes patients cannot make enough insulin. However, beta-cell function could be improved by food with low postprandial glucose through (1) reducing glucose toxicity on body tissues and regulatory processes, (2) reducing serum-free fatty acids, which is related to insulin secretion, and (3) increasing the levels of incretion hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), which may be responsible for satiety and less energy intake (Wolever, 2013). Significantly lower glycemic responses at early time points after food ingestion or lower incremental area under glucose concentration curve were reported in a few human dietary intervention studies using high-amylose maize starch (Anderson et al., 2010; Behall & Scholfield, 2005; Brighenti et al., 2006).

The undigested fraction of HAS showed enhanced fermentation activity in rats (Conlon et al., 2012; Hazard et al., 2015). The fermented products of RS such as short-chain fatty acids (SCFAs) have been demonstrated to have bowel health benefits, including reducing the likelihood of colorectal cancer and improvement of insulin responses (Topping & Clifton, 2001). The RS as growth substrate in the large colon is degraded by the human colonic microbiota in which *Firmicutes* and *Bacteroidetes* are the two most abundant bacteria phyla. Human diets high in RS have increased fecal levels of *Ruminococcus bromii* and *Eubacterium rectale* (both in the phylum *Firmicutes*) in overweight males (Walker et al., 2010). However, depending on the nature of RS, 3 distinct microbial
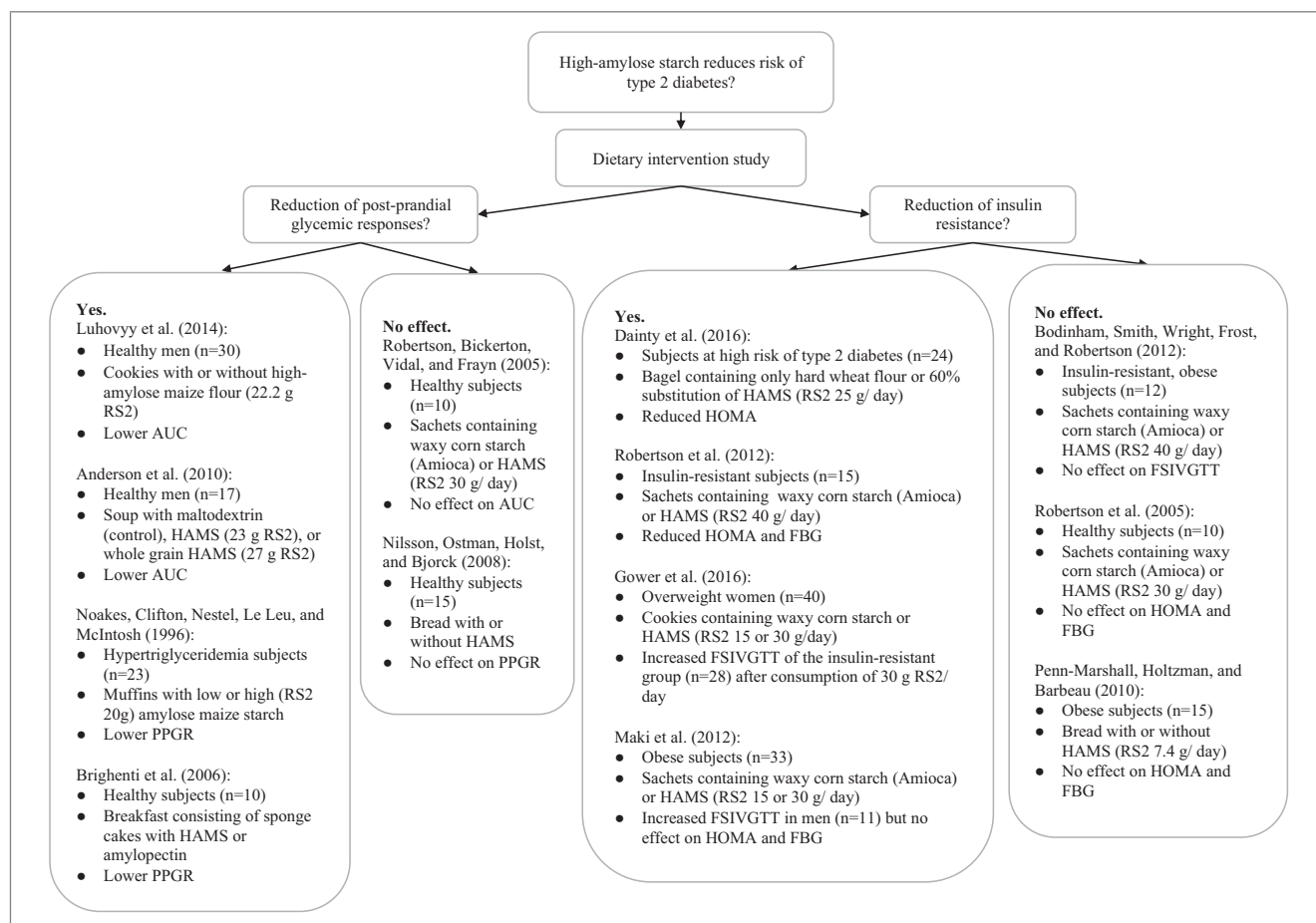
Figure 6–Dietary intervention studies on high-amylose starch and risk reduction of type 2 diabetes. AUC: postprandial glycemic area under the curve; HAMS: high-amylose maize starch; HOMA: homeostasis model assessment for insulin resistance; FBG: fasting blood sugar level; FSIVGTT: frequently sampled intravenous glucose tolerance test for insulin sensitivity; PPGR: postprandial glucose response.

communities resulting from *in vitro* fermentation using a porcine fecal inoculum were identified (Warren et al., 2018). Fermentation of HAS potentially modifies the population of the microbiota that underlies colonic metabolism and the body immune system. It is also possible that different physicochemical structures of the fermentable substrate may favor the growth of specific gut bacterial species in competitive niches of the colon. A greater understanding of the relationships between the population shift of the microbiota and HAS structure would allow for optimal selection of HAS as dietary fibers for colonic health.

Conclusions and Future Perspectives

Starch is the principal component of grains and tubers. A wide range of high-amylose versions of major starchy food crops is now available, which will allow a better selection of cereal/tuber varieties with enhanced human nutrition and desired functional properties. HAS is important for human nutrition because of its contribution to intake of dietary fiber. The starch molecular structure can be tailored through the selection of biotechnological approaches to elevate amylose content, which, in turn, modify the multilevel starch structures. A greater understanding of how starch structural features enhance RS content and affect food application properties can be helpful in designing nutrition-enhanced grains without compromising food sensory quality in food applications. The native granular structures of HAS generally have reduced digestibility compared to the corresponding regular starch.

Furthermore, HAS tends to retain or form dense molecular structures during food processing that are mostly resistant to amylase digestion. While there is good understanding of biological and structural aspects of HAS, the time has now come to evaluate options to incorporate HAS with enhanced nutritional value in food products. The development and near commercialization of high-amylose wheat flour will certainly open an avenue to develop multiple wheat-based products with high-amylose wheat flour or wholegrains. This will shift the HAS from auxiliary ingredient (for example, substitute of wheat flour) to major ingredient (replacement of regular wheat flour). The following are some recommendations for future studies.

(1) There is only limited information about the breakdown properties of HAS in relation to the whole diet in the human gastrointestinal tract. Though there is extensive knowledge about the slow *in vitro* digestibility of HAS, future work is required to explore its nutritional effect in terms of both lowering the glycemic response and improving colonic health when in real food, as well as elucidating the causative mechanisms underlying the correlation between physicochemical structures and metabolic responses.

(2) The applicability of high-amylose wheat flour as a replacement of common bread and pasta flour depends on the quality and quantity of gluten. Further work is needed to understand the change in protein (gluten) profile (quantity

and quality) with respect to increases in amylose content in the grain.

Authors' Contributions

Haiteng Li as 1st author designed and wrote the manuscript with critical input from Michael J. Gidley and Sushil Dhital.

Conflicts of Interest

The authors have declared no conflict of interest.

References

Ai, Y., Hasjim, J., & Jane, J.-L. (2013). Effects of lipids on enzymatic hydrolysis and physical properties of starch. *Carbohydrate Polymers*, *92*(1), 120–127.

Al-Rabadi, G. J. S., Gilbert, R. G., & Gidley, M. J. (2009). Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase. *Journal of Cereal Science*, *50*(2), 198–204.

Anderson, G. H., Cho, C. E., Akhavan, T., Mollard, R. C., Luhovyy, B. L., & Finocchiaro, E. T. (2010). Relation between estimates of cornstarch digestibility by the Englyst *in vitro* method and glycemic response, subjective appetite, and short-term food intake in young men. *American Journal of Clinical Nutrition*, *91*(4), 932–939.

Aravind, N., Sissons, M., Fellows, C. M., Blazek, J., & Gilbert, E. P. (2013). Optimisation of resistant starch II and III levels in durum wheat pasta to reduce *in vitro* digestibility while maintaining processing and sensory characteristics. *Food Chemistry*, *136*(2), 1100–1109.

Baik, B. K., & Lee, M. R. (2003). Effects of starch amylose content of wheat on textural properties of white salted noodles. *Cereal Chemistry*, *80*(3), 304–309.

Ball, S., Guan, H.-P., James, M., Myers, A., Keeling, P., Mouille, G., ... Preiss, J. (1996). From glycogen to amylopectin: A model for the biogenesis of the plant starch granule. *Cell*, *86*(3), 349–352.

Bandillo, N., Raghavan, C., Muyco, P. A., Sevilla, M. A. L., Lobina, I. T., Dilla-Ermita, C. J., ... Leung, H. J. R. (2013). Multi-parent advanced generation inter-cross (MAGIC) populations in rice: Progress and potential for genetics research and breeding. *Rice*, *6*(1), 11.

Becker, A., Hill, S. E., & Mitchell, J. R. (2001). Relevance of amylose-lipid complexes to the behaviour of thermally processed starches. *Starch-Stärke*, *53*(3–4), 121–130.

Behall, K., & Scholfield, D. (2005). Food amylose content affects postprandial glucose and insulin responses. *Cereal Chemistry*, *82*(6), 654–659.

Bertoft, E. (2004). On the nature of categories of chains in amylopectin and their connection to the super helix model. *Carbohydrate Polymers*, *57*(2), 211–224.

Bertoft, E. (2013). On the building block and backbone concepts of amylopectin structure. *Cereal Chemistry*, *90*(4), 294–311.

Bertoft, E. (2017). Understanding starch structure: Recent progress. *Agronomy Journal*, *7*(3), 56.

Bird, A. R., & Regina, A. (2018). High amylose wheat: A platform for delivering human health benefits. *Journal of Cereal Science*, *82*, 99–105.

Blauth, S. L., Kim, K.-N., Klucinec, J., Shannon, J. C., Thompson, D., & Guiltinan, M. (2002). Identification of *Mutator* insertional mutants of starch-branching enzyme 1 (*sbe1*) in *Zea mays* L. *Plant Molecular Biology*, *48*(3), 287–297.

Blazek, J., Salman, H., Rubio, A. L., Gilbert, E., Hanley, T., & Copeland, L. (2009). Structural characterization of wheat starch granules differing in amylose content and functional characteristics. *Carbohydrate Polymers*, *75*(4), 705–711.

Bodinham, C. L., Smith, L., Wright, J., Frost, G. S., & Robertson, M. D. (2012). Dietary fibre improves first-phase insulin secretion in overweight individuals. *PLoS One*, *7*(7), e40834.

Brighenti, F., Benini, L., Del Rio, D., Casiraghi, C., Pellegrini, N., Scazzina, F. D. J. A., & Vantini, I. (2006). Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. *American Journal of Clinical Nutrition*, *83*(4), 817–822.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules*, *23*(2), 85–112.

Butardo, V. M., Anacleto, R., Parween, S., Samson, I., de Guzman, K., Alhambra, C. M., ... Sreenivasulu, N. (2017). Systems genetics identifies a novel regulatory domain of amylose synthesis. *Plant Physiology*, *173*(1), 887–906.

Butardo, V. M., Fitzgerald, M. A., Bird, A. R., Gidley, M. J., Flanagan, B. M., Larroque, O., ... Rahman, S. (2011). Impact of down-regulation of starch branching enzyme IIb in rice by artificial micro RNA- and hairpin RNA- mediated RNA silencing. *Journal of Experimental Botany*, *62*(14), 4927–4941.

Cai, L., & Shi, Y.-C. (2010). Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches. *Carbohydrate Polymers*, *79*(4), 1117–1123.

Cai, L., & Shi, Y.-C. (2013). Self-assembly of short linear chains to A- and B-type starch spherulites and their enzymatic digestibility. *Journal of Agricultural and Food Chemistry*, *61*(45), 10787–10797.

Cai, L., Shi, Y.-C., Rong, L., & Hsiao, B. S. (2010). Debranching and crystallization of waxy maize starch in relation to enzyme digestibility. *Carbohydrate Polymers*, *81*(2), 385–393.

Campbell, M. R., White, P. J., & Pollak, L. M. (1994). Dosage effect at the sugary-2 locus on maize starch structure and function. *Cereal Chemistry*, *71*(5), 464–468.

Carciofi, M., Blennow, A., Jensen, S., Shaik, S., Henriksen, A., Buléon, A., ... Hebelstrup, K. (2012). Concerted suppression of all starch branching enzyme genes in barley produces amylose-only starch granules. *BMC Plant Biology*, *12*, 223.

Cavanagh, C., Morell, M., Mackay, I., & Powell, W. (2008). From mutations to MAGIC: Resources for gene discovery, validation and delivery in crop plants. *Current Opinion in Plant Biology*, *11*(2), 215–221.

Colleoni, C., Myers, A. M., & James, M. G. (2003). One- and two-dimensional native PAGE activity gel analyses of maize endosperm proteins reveal functional interactions between specific starch metabolizing enzymes. *Journal of Applied Glycoscience*, *50*(2), 207–212.

Conlon, M. A., Kerr, C. A., McSweeney, C. S., Dunne, R. A., Shaw, J. M., Kang, S., ... Regina, A. (2012). Resistant starches protect against colonic DNA damage and alter microbiota and gene expression in rats fed a western diet. *Journal of Nutrition*, *142*(5), 832–840.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: Origin of the enthalpic transition. *Carbohydrate Research*, *227*(Supplement C), 103–112.

Craig, J., Lloyd, J. R., Tomlinson, K., Barber, L., Edwards, A., Wang, T. L., ... Smith, A. M. (1998). Mutations in the gene encoding starch synthase II profoundly alter amylopectin structure in pea embryos. *Plant Cell*, *10*(3), 413–426.

Cui, R., & Oates, C. G. (1999). The effect of amylose–lipid complex formation on enzyme susceptibility of sago starch. *Food Chemistry*, *65*(4), 417–425.

Dainty, S. A., Klingel, S. L., Pilkey, S. E., McDonald, E., McKeown, B., Emes, M. J., & Duncan, A. M. (2016). Resistant starch bagels reduce fasting and postprandial insulin in adults at risk of type 2 diabetes. *Journal of Nutrition*, *146*(11), 2252–2259.

Dhital, S., Bhattarai, R. R., Gorham, J., & Gidley, M. J. (2016). Intactness of cell wall structure controls the in vitro digestion of starch in legumes. *Food & Function*, *7*(3), 1367–1379.

Dhital, S., Butardo, V. M., Jobling, S. A., & Gidley, M. J. (2015). Rice starch granule amylolysis – Differentiating effects of particle size, morphology, thermal properties and crystalline polymorph. *Carbohydrate Polymers*, *115*, 305–316.

Dhital, S., Dolan, G., Stokes, J. R., & Gidley, M. J. (2014). Enzymatic hydrolysis of starch in the presence of cereal soluble fibre polysaccharides. *Food & Function*, *5*(3), 579–586.

Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and *in vitro* digestibility of maize and potato starches. *Carbohydrate Polymers*, *82*(2), 480–488.

Dhital, S., Warren, F. J., Butterworth, P. J., Ellis, P. R., & Gidley, M. J. (2017). Mechanisms of starch digestion by α-amylase-structural basis for kinetic properties. *Critical Reviews in Food Science and Nutrition*, *57*(5), 875–892.

Dinges, J. R., Colleoni, C., James, M. G., & Myers, A. M. (2003). Mutational analysis of the pullulanase-type debranching enzyme of maize indicates multiple functions in starch metabolism. *Plant Cell*, *15*(3), 666–680.

Dona, A. C., Pages, G., Gilbert, R. G., & Kuchel, P. W. (2010). Digestion of starch: *In vivo* and *in vitro* kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers*, 80(3), 599–617.

Edwards, A., Fulton, D. C., Hylton, C. M., Jobling, S. A., Gidley, M., Rössner, U., . . . Smith, A. M. (1999). A combined reduction in activity of starch synthases II and III of potato has novel effects on the starch of tubers. *Plant Journal*, 17(3), 251–261.

Edwards, C. H., Grundy, M. M. L., Grassby, T., Vasilopoulou, D., Frost, G. S., Butterworth, P. J., . . . Ellis, P. R. (2015). Manipulation of starch bioaccessibility in wheat endosperm to regulate starch digestion, postprandial glycemia, insulinemia, and gut hormone responses: A randomized controlled trial in healthy ileostomy participants. *American Journal of Clinical Nutrition*, 102(4), 791–800.

Edwards, C. H., Warren, F. J., Milligan, P. J., Butterworth, P. J., & Ellis, P. R. (2014). A novel method for classifying starch digestion by modelling the amylolysis of plant foods using first-order enzyme kinetic principles. *Food & Function*, 5(11), 2751–2758.

EFSA. (2011). Scientific opinion on the substantiation of health claims related to resistant starch and reduction of post-prandial glycaemic responses (ID 681), "digestive health benefits" (ID 682) and "favours a normal colon metabolism" (ID 783) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal*, 9(4), 2024.

Ellis, P. R., Kendall, C. W. C., Ren, Y., Parker, C., Pacy, J. F., Waldron, K. W., & Jenkins, D. J. A. (2004). Role of cell walls in the bioaccessibility of lipids in almond seeds. *American Journal of Clinical Nutrition*, 80(3), 604–613.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46(Suppl 2), S33–50.

Englyst, H. N., Kingman, S. M., Hudson, G. J., & Cummings, J. H. (1996). Measurement of resistant starch *in vitro* and *in vivo*. *British Journal of Nutrition*, 75(5), 749–755.

Evans, A., & Thompson, D. (2004). Resistance to α-amylase digestion in four native high-amylose maize starches. *Cereal Chemistry*, 81(1), 31–37.

Fitzgerald, M. A., Bergman, C. J., Resurreccion, A. P., Moller, J., Jimenez, R., Reinke, R., . . . Mestres, C. (2009). Addressing the dilemmas of measuring amylose in rice. *Cereal Chemistry*, 86(5), 492–498.

Flipse, E., Keetels, C., Jacobsen, E., & Visser, R. (1996). The dosage effect of the wildtype GBSS allele is linear for GBSS activity but not for amylose content: Absence of amylose has a distinct influence on the physico-chemical properties of starch. *International Journal of Plant Breeding Research*, 92(1), 121–127.

Fujita, N., Yoshida, M., Asakura, N., Ohdan, T., Miyao, A., Hirochika, H., & Nakamura, Y. (2006). Function and characterization of starch synthase I using mutants in rice. *Plant Physiology*, 140(3), 1070–1084.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers*, 32(3), 177–191.

Gidley, M. J. (1987). Factors affecting the crystalline type (A–C) of native starches and model compounds: A rationalisation of observed effects in terms of polymorphic structures. *Carbohydrate Research*, 161(2), 301–304.

Gidley, M. J., & Bulpin, P. V. (1987). Crystallisation of malto–oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydrate Research*, 161(2), 291–300.

Gidley, M. J., & Bulpin, P. V. (1989). Aggregation of amylose in aqueous systems: The effect of chain length on phase behavior and aggregation kinetics. *Macromolecules*, 22(1), 341–346.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: How they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology*, 16(1), 12–30.

Gower, B., Bergman, R., Stefanovski, D., Darnell, B., Ovalle, F., Fisher, G., . . . Pelkman, C. (2016). Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: A randomized, controlled trial. *Nutrition & Metabolism*, 13, 2.

Grassby, T., Edwards, C., Grundy, M., & Ellis, P. (2013). Functional components and mechanisms of action of 'dietary fibre' in the upper gastrointestinal tract. In S. E. Harding (Ed.), *Stability of complex carbohydrate structures* (pp. 36–55). Cambridge: The Royal Society of Chemistry.

Graybosch, R., Peterson, C. J., Moore, K. J., Stearns, M., & Grant, D. L. (1993). Comparative effects of wheat flour protein, lipid, and pentosan composition in relation to baking and milling quality. *Cereal Chemistry*, 70, 95–101.

Grundy, M. M. L., Edwards, C. H., Mackie, A. R., Gidley, M. J., Butterworth, P. J., & Ellis, P. R. (2016). Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition*, 116, 816–833.

Guo, G., Jackson, D., Graybosch, R., & Parkhurst, A. (2003). Asian salted noodle quality: Impact of amylose content adjustments using waxy wheat flour. *Cereal Chemistry*, 80(4), 437–445.

Hallauer, A. R. (2001). *Specialty corns* (2nd ed.). Boca Raton, FL: CRC Press.

Hanashiro, I., Abe, J.-I., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, 283(Supplement C), 151–159.

Hasjim, J., Ai, Y., & Jane, J.-l. (2013). Novel applications of amylose-lipid complex as resistant starch type 5. In Y. C. Shi & C. C. Maningat (Eds.), *Resistant starch: Sources, applications and health benefits* (pp. 79–94). Chichester, UK: John Wiley and Sons Ltd.

Hasjim, J., Lee, S.-O., Hendrich, S., Setiawan, S., Ai, Y., & Jane, J.-L. (2010). Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. *Cereal Chemistry*, 87(4), 257–262.

Hazard, B., Zhang, X., Naemeh, M., Hamilton, M. K., Rust, B., Raybould, H. E., . . . Dubcovsky, J. (2015). Mutations in durum wheat genes affect grain yield components, quality, and fermentation responses in rats. *Crop Science*, 55(6), 2813–2825.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research*, 147(2), 342–347.

Hoebler, C., Karinthi, A., Chiron, H., Champ, M., & Barry, J. L. (1999). Bioavailability of starch in bread rich in amylose: Metabolic responses in healthy subjects and starch structure. *European Journal of Clinical Nutrition*, 53(5), 360–366.

Hofvander, P., Andersson, M., Larsson, C. T., & Larsson, H. (2004). Field performance and starch characteristics of high-amylose potatoes obtained by antisense gene targeting of two branching enzymes. *Plant Biotechnology Journal*, 2(4), 311–320.

Huang, J., Shang, Z., Man, J., Liu, Q., Zhu, C., & Wei, C. (2015). Comparison of molecular structures and functional properties of high-amylose starches from rice transgenic line and commercial maize. *Food Hydrocolloids*, 46, 172–179.

IOM. (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Washington, DC: National Academies Press.

Itoh, K., Ozaki, H., Okada, K., Hori, H., Takeda, Y., & Mitsui, T. (2003). Introduction of *wx* transgene into rice *wx* mutants leads to both high- and low-amylose rice. *Plant and Cell Physiology*, 44(5), 473–480.

Jane, J.-L. (2006). Current understanding on starch granule structures. *Journal of Applied Glycoscience*, 53(3), 205–213.

Jane, J.-L. (2009). Structural features of starch granules II–chapter 6. In R. L. Whistler, James N. Bemiller, & E. P. Paschall (Eds.), *Starch: Chemistry and technology* (3rd ed., pp. 193–236). Amsterdam/New York: Elsevier Inc.

Jane, J.-L., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, 76(5), 629–637.

Jane, J.-L., & Robyt, J. F. (1984). Structure studies of amylose-V complexes and retro-graded amylose by action of alpha amylases, and a new method for preparing amylodextrins. *Carbohydrate Research*, 132(1), 105–118.

Jane, J.-L., & Shen, J. (1993). Internal structure of the potato starch granule revealed by chemical gelatinization. *Carbohydrate Research*, 247, 279–290.

Jiang, H., Campbell, M., Blanco, M., & Jane, J.-L. (2010). Characterization of maize amylose-extender (*ae*) mutant starches: Part II. Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature. *Carbohydrate Polymers*, 80(1), 1–12.

Jiang, H., Horner, H. T., Pepper, T. M., Blanco, M., Campbell, M., & Jane, J.-L. (2010). Formation of elongated starch granules in high-amylose maize. *Carbohydrate Polymers*, 80(2), 533–538.

Jobling, S. A., Schwall, G. P., Westcott, R. J., Sidebottom, C. M., Debet, M., Gidley, M. J., . . . Safford, R. (1999). A minor form of starch branching enzyme in potato (*Solanum tuberosum* L.) tubers has a major effect on starch structure: Cloning and characterisation of multiple forms of SBE A. *Plant Journal*, 18(2), 163–171.

Kasemsuwan, T., Jane, J., Schnable, P., Stinard, P., & Robertson, D. (1995). Characterization of the dominant mutant amylose-extender (*Ae1-5180*) maize starch. *Cereal Chemistry*, 72(5), 457–464.

Konik-Rose, C., Thistleton, J., Chanvrier, H., Tan, I., Halley, P., Gidley, M., . . . Li, Z. (2007). Effects of starch synthase IIa gene dosage on grain, protein and starch in endosperm of wheat. *Theoretical and Applied Genetics*, *115*(8), 1053–1065.

Larsson, H., & Eliasson, A. C. (1997). Influence of the starch granule surface on the rheological behaviour of wheat flour dough. *Journal of Texture Studies*, *28*(5), 487–501.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., & Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers*, *18*(3), 189–197.

Li, L., Jiang, H., Campbell, M., Blanco, M., & Jane, J.-L. (2008). Characterization of maize amylose-extender (*ae*) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydrate Polymers*, *74*(3), 396–404.

Li, M., Dhital, S., & Wei, Y. (2017). Multilevel structure of wheat starch and its relationship to noodle eating qualities. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1042–1055.

Liu, H., Yu, L., Simon, G., Zhang, X., Dean, K., & Chen, L. (2009). Effect of annealing and pressure on microstructure of cornstarches with different amylose/amylopectin ratios. *Carbohydrate Research*, *344*(3), 350–354.

Lockyer, S., & Nugent, A. P. (2017). Health effects of resistant starch. *Nutrition Bulletin*, *42*(1), 10–41.

Luhovyy, B. L., Mollard, R. C., Yurchenko, S., Nunez, M. F., Berengut, S., Liu, T. T., . . . Anderson, G. H. (2014). The effects of whole grain high-amylose maize flour as a source of resistant starch on blood glucose, satiety, and food intake in young men. *Journal of Food Science*, *79*(12), H2550–H2556.

Maki, K., Pelkman, C., Finocchiaro, E., Kelley, K., Lawless, A., Schild, A., & Rains, T. (2012). Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *Journal of Nutrition*, *142*(4), 717–723.

McCallum, C. M., Comai, L., Greene, E. A., & Henikoff, S. (2000). Targeting induced local lesions IN Genomes (TILLING) for plant functional genomics. *Plant Physiology*, *123*(2), 439–442.

McCleary, B. V. (2007). An integrated procedure for the measurement of total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates. *Analytical and Bioanalytical Chemistry*, *389*(1), 291–308.

McCleary, B. V. (2013). Measurement of resistant starch and incorporation of resistant starch into dietary fibre measurements. In Y. C. Shi & C. C. Maningat (Eds.), *Resistant starch: Sources, applications and health benefits* (pp. 131–144). Chichester, UK: John Wiley and Sons Ltd.

McPherson, A. E., & Jane, J.-L. (1999). Comparison of waxy potato with other root and tuber starches. *Carbohydrate Polymers*, *40*(1), 57–70.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*(2), 271–281.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., . . . Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food an international consensus. *Food & Function*, *5*(6), 1113–1124.

Morell, M. K., Kosar-Hashemi, B., Cmiel, M., Samuel, M. S., Chandler, P., Rahman, S., . . . Li, Z. (2003). Barley *sex6* mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant Journal*, *34*(2), 173–185.

Morita, N., Maeda, T., Miyazaki, M., Yamamori, M., Miura, H., & Ohtsuka, I. (2002). Dough and baking properties of high-amylose and waxy wheat flours. *Cereal Chemistry*, *79*(4), 491–495.

Morrison, W. R. (1988). Lipids in cereal starches: A review. *Journal of Cereal Science*, *8*(1), 1–15.

Morrison, W. R., Tester, R. F., Snape, C. E., Law, R., & Gidley, M. J. (1993). Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. *Cereal Chemistry*, *70*(4), 385–391.

Myers, A. M., Morell, M. K., James, M. G., & Ball, S. G. (2000). Recent progress toward understanding the amylopectin crystal. *Plant Physiology*, *122*(4), 989–997.

Nakamura, Y. (2018). Rice starch biotechnology: Rice endosperm as a model of cereal endosperms. *Starch-Stärke*, *70*(1–2), 1600375.

Nakamura, Y., Utsumi, Y., Sawada, T., Aihara, S., Utsumi, C., Yoshida, M., & Kitamura, S. (2010). Characterization of the reactions of starch branching enzymes from rice endosperm. *Plant and Cell Physiology*, *51*(5), 776–794.

Nichols, B. L., Avery, S., Sen, P., Swallow, D. M., Hahn, D., & Sterchi, E. (2003). The maltase-glucoamylase gene: common ancestry to sucrase-isomaltase with complementary starch digestion activities. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 1432–1437.

Nilsson, A. C., Ostman, E. M., Holst, J. J., & Bjorck, I. M. E. (2008). Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. *Journal of Nutrition*, *138*(4), 732–739.

Nishi, A., Nakamura, Y., Tanaka, N., & Satoh, H. (2001). Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. *Plant Physiology*, *127*(2), 459–472.

Noah, L., Guillon, F., Bouchet, B., Buleon, A., Molis, C., Gratas, M., & Champ, M. (1998). Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L.) in healthy humans. *Journal of Nutrition*, *128*(6), 977–985.

Noakes, M., Clifton, P. M., Nestel, P. J., Le Leu, R., & McIntosh, G. (1996). Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. *American Journal of Clinical Nutrition*, *64*(6), 944–951.

Nugent, A. P. (2005). Health properties of resistant starch. *Nutrition Bulletin*, *30*, 27–54.

Pan, D. D., & Jane, J.-L. (2000). Internal structure of normal maize starch granules revealed by chemical surface gelatinization. *Biomacromolecules*, *1*(1), 126–132.

Penn-Marshall, M., Holtzman, G. I., & Barbeau, W. E. (2010). African Americans may have to consume more than 12 grams a day of resistant starch to lower their risk for type 2 diabetes. *Journal of Medicinal Food*, *13*(4), 999–1004.

Pérez, S., Baldwin, P. M., & Gallant, D. J. (2009). Structural features of starch granules I-chapter 5. In R. L. Whistler, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch: Chemistry and technology* (3rd ed., pp. 149–192). Amsterdam/New York: Elsevier Inc.

Planchot, V., Colonna, P., & Buleon, A. (1997). Enzymatic hydrolysis of α-glucan crystallites. *Carbohydrate Research*, *298*(4), 319–326.

Regina, A., Berbezy, P., Kosar-Hashemi, B., Li, S., Cmiel, M., Larroque, O., . . . Morell, M. (2015). A genetic strategy generating wheat with very high amylose content. *Plant Biotechnology Journal*, *13*(9), 1276–1286.

Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., Barsby, T., . . . Morell, M. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(10), 3546–3551.

Regina, A., Blazek, J., Gilbert, E., Flanagan, B. M., Gidley, M. J., Cavanagh, C., . . . Morell, M. K. (2012). Differential effects of genetically distinct mechanisms of elevating amylose on barley starch characteristics. *Carbohydrate Polymers*, *89*(3), 979–991.

Regina, A., Kosar-Hashemi, B., Li, Z., Rampling, L., Cmiel, M., Gianibelli, M. C., . . . Morell, M. K. (2004). Multiple isoforms of starch branching enzyme-I in wheat: Lack of the major SBE-I isoform does not alter starch phenotype. *Functional Plant Biology*, *31*(6), 591–601.

Regina, A., Kosar-Hashemi, B., Ling, S., Li, Z., Rahman, S., & Morell, M. (2010). Control of starch branching in barley defined through differential RNAi suppression of starch branching enzyme IIa and IIb. *Journal of Experimental Botany*, *61*(5), 1469–1482.

Ring, S. G., L'Anson, K., & Morris, V. J. (1985). Static and dynamic light scattering studies of amylose solutions. *Macromolecules*, *18*(2), 182–188.

Robertson, M. D., Bickerton, A. S., Vidal, H., & Frayn, K. N. (2005). Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *American Journal of Clinical Nutrition*, *82*(3), 559–567.

Robertson, M. D., Wright, J. W., Loizon, E., Debard, C., Vidal, H., Shojaee-Moradie, F., . . . Umpleby, A. M. (2012). Insulin-sensitizing effects on muscle and adipose tissue after dietary fiber intake in men and women with metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism*, *97*(9), 3326–3332.

Ryoo, N., Yu, C., Park, C.-S., Baik, M.-Y., Park, I., Cho, M.-H., . . . Jeon, J.-S. (2007). Knockout of a starch synthase gene *OsSSIIIa / Flo5* causes white-core floury endosperm in rice (*Oryza sativa* L.). *Plant Cell Reports*, *26*(7), 1083–1095.

Satoh, H., Nishi, A., Yamashita, K., & Takemoto, Y. (2003). Starch-branching enzyme I-deficient mutation specifically affects the structure and properties of starch in rice endosperm1. *Plant Physiology*, *133*(3), 1111–1121.

Schonhofen, A., Hazard, B., Zhang, X., & Dubcovsky, J. (2016). Registration of common wheat germplasm with mutations in SBEII genes conferring increased grain amylose and resistant starch content. *Journal of Plant Registration*, *10*(2), 200–205.

Schwall, G. P., Safford, R., Westcott, R. J., Jeffcoat, R., Tayal, A., Yong-Cheng, S., . . . Jobling, S. A. (2000). Production of very-high-amylose potato starch by inhibition of SBE A and B. *Nature Biotechnology*, *18*(5), 551–554.

Sestili, F., Botticella, E., Proietti, G., Janni, M., D'ovidio, R., & Lafiandra, D. (2012). Amylose content is not affected by overexpression of the *Wx-B1* gene in durum wheat. *Plant Breeding*, *131*(6), 700–706.

Shanahan, F., van Sinderen, D., O'Toole, P. W., & Stanton, C. (2017). Feeding the microbiota: Transducer of nutrient signals for the host. *Gut*, *66*(9), 1709–1717.

Shi, Y.-C., Capitani, T., Trzasko, P., & Jeffcoat, R. (1998). Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *Journal of Cereal Science*, *27*(3), 289–299.

Shimbata, T., Ai, Y., Fujita, M., Inokuma, T., Vrinten, P., Sunohara, A., . . . Nakamura, T. (2012). Effects of homoeologous wheat starch synthase IIa genes on starch properties. *Journal of Agricultural and Food Chemistry*, *60*(48), 12004–12010.

Shrestha, A. K., Blazek, J., Flanagan, B. M., Dhital, S., Larroque, O., Morell, M. K., . . . Gidley, M. J. (2015). Molecular, mesoscopic and microscopic structure evolution during amylase digestion of extruded maize and high amylose maize starches. *Carbohydrate Polymers*, *118*, 224–234.

Slade, A. J., Fuerstenberg, S. I., Loeffler, D., Steine, M. N., & Facciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature Biotechnology*, *23*(1), 75–81.

Slade, A. J., McGuire, C., Loeffler, D., Mullenberg, J., Skinner, W., Fazio, G., . . . Knauf, V. C. (2012). Development of high amylose wheat through TILLING. *BMC Plant Biology*, *12*, 69.

Soh, H. N., Sissons, M. J., & Turner, M. A. (2006). Effect of starch granule size distribution and elevated amylose content on dorum dough rheology and spaghetti cooking quality. *Cereal Chemistry*, *83*(5), 513–519.

Tester, R. F., Qi, X., & Karkalas, J. (2006). Hydrolysis of native starches with amylases. *Animal Feed Science and Technology*, *130*, 39–54.

Tetlow, I. J. (2011). Starch biosynthesis in developing seeds. *Seed Science Research*, *21*(1), 5–32.

Tetlow, I. J., & Emes, M. J. (2014). A review of starch-branching enzymes and their role in amylopectin biosynthesis. *IUBMB Life*, *66*(8), 546–558.

Tetlow, I. J., Morell, M. K., & Emes, M. J. (2004). Recent developments in understanding the regulation of starch metabolism in higher plants. *Journal of Experimental Botany*, *55*(406), 2131–2145.

Tetlow, I. J., Wait, R., Lu, Z., Akkasaeng, R., Bowsher, C. G., Esposito, S., . . . Emes, M. J. (2004). Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. *Plant Cell*, *16*(3), 694–708.

Tomlinson, K., & Denyer, K. (2003). Starch synthesis in cereal grains. *Advances in Botanical Research*, *40*, 1–61.

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, *81*(3), 1031–1064.

Tovar, J., Bjorck, I. M., & Asp, N. G. (1992). Incomplete digestion of legume starches in rats: A study of precooked flours containing retrograded and physically inaccessible starch fractions. *Journal of Nutrition*, *122*(7), 1500–1507.

Umemoto, T., Aoki, N., Lin, H., Nakamura, Y., Inouchi, N., Sato, Y., . . . Maruyama, S. (2004). Natural variation in rice starch synthase IIa affects enzyme and starch properties. *Functional Plant Biology*, *31*(7), 671–684.

Umemoto, T., Horibata, T., Aoki, N., Hiratsuka, M., Yano, M., & Inouchi, N. (2008). Effects of variations in starch synthase on starch properties and eating quality of rice. *Plant Production Science*, *11*(4), 472–480.

Valentina, T., & Fredrik, B. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature*, *489*(7415), 242–249.

Van Hung, P., Maeda, T., & Morita, N. (2006). Waxy and high-amylose wheat starches and flours—Characteristics, functionality and application. *Trends in Food Science & Technology*, *17*(8), 448–456.

Van Hung, P., Yamamori, M., & Morita, N. (2005). Formation of enzyme-resistant starch in bread as affected by high-amylose wheat flour substitutions. *Cereal Chemistry*, *82*(6), 690–694.

Vilaplana, F., Hasjim, J., & Gilbert, R. G. (2012). Amylose content in starches: Toward optimal definition and validating experimental methods. *Carbohydrate Polymers*, *88*(1), 103–111.

Vilaplana, F., Meng, D., Hasjim, J., & Gilbert, R. G. (2014). Two-dimensional macromolecular distributions reveal detailed architectural features in high-amylose starches. *Carbohydrate Polymers*, *113*, 539–551.

Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., . . . Flint, H. J. (2010). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME Journal*, *5*(2), 220–230.

Wang, J., Hu, P., Chen, Z., Liu, Q., & Wei, C. (2017). Progress in high-amylose cereal crops through inactivation of starch branching enzymes. *Frontiers in Plant Science*, *8*, 469.

Wang, T., Bogracheva, T., & Hedley, C. (1998). Starch: As simple as A, B, C? *Journal of Experimental Botany*, *49*, 481–502.

Warren, F. J., Fukuma, N. M., Mikkelsen, D., Flanagan, B. M., Williams, B. A., Lisle, A. T., . . . Gidley, M. J. (2018). Food starch structure impacts gut microbiome composition. *mSphere*, *3*(3), e00086–00018.

Wei, C., Qin, F., Zhu, L., Zhou, W., Chen, Y., Wang, Y., . . . Liu, Q. (2010). Microstructure and ultrastructure of high-amylose rice resistant starch granules modified by antisense RNA inhibition of starch branching enzyme. *Journal of Agricultural and Food Chemistry*, *58*(2), 1224–1232.

Wei, C., Xu, B., Qin, F., Yu, H., Chen, C., Meng, X., . . . Liu, Q. (2010). C-type starch from high-amylose rice resistant starch granules modified by antisense RNA inhibition of starch branching enzyme. *Journal of Agricultural and Food Chemistry*, *58*(12), 7383–7888.

Williamson, G., Belshaw, N. J., Self, D. J., Noel, T. R., Ring, S. G., Cairns, P., . . . Parker, M. L. (1992). Hydrolysis of A- and B-type crystalline polymorphs of starch by α-amylase, β-amylase and glucoamylase. *Carbohydrate Polymers*, *18*(3), 179–187.

Witt, T., Gidley, M. J., & Gilbert, R. G. (2010). Starch digestion mechanistic information from the time evolution of molecular size distributions. *Journal of Agricultural and Food Chemistry*, *58*(14), 8444–8452.

Wolever, T. M. S. (2013). Role of carbohydrates in the prevention of type 2 diabetes. In Y. C. Shi & C. C. Maningat (Eds.), *Resistant starch: Sources, applications and health benefits* (pp. 191–205). Chichester, UK: John Wiley and Sons Ltd.

Wu, A. C., Li, E., & Gilbert, R. G. (2014). Exploring extraction/dissolution procedures for analysis of starch chain-length distributions. *Carbohydrate Polymers*, *114*, 36–42.

Yamamori, M., Fujita, S., Hayakawa, K., Matsuki, J., & Yasui, T. (2000). Genetic elimination of a starch granule protein, SGP-1, of wheat generates an altered starch with apparent high amylose. *International Journal of Plant Breeding Research*, *101*(1), 21–29.

Yoo, S.-H., & Jane, J.-L. (2002). Structural and physical characteristics of waxy and other wheat starches. *Carbohydrate Polymers*, *49*(3), 297–305.

Zeeman, S. C., Kossmann, J., & Smith, A. M. (2010). Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, *61*, 209–234.

Zhang, B., Dhital, S., & Gidley, M. J. (2015). Densely packed matrices as rate determining features in starch hydrolysis. *Trends in Food Science & Technology*, *43*(1), 18–31.

Zhang, G., Ao, Z., & Hamaker, B. (2006). Slow digestion property of native cereal starches. *Biomacromolecules*, *7*(11), 3252–3258.

Zhang, G., Ao, Z., & Hamaker, B. R. (2008). Nutritional property of endosperm starches from maize mutants: A parabolic relationship between slowly digestible starch and amylopectin fine structure. *Journal of Agricultural and Food Chemistry*, *56*(12), 4686–4694.

Zhang, J., & Datta, A. K. (2006). Mathematical modeling of bread baking process. *Journal of Food Engineering*, *75*(1), 78–89.

Zhang, X., Colleoni, C., Ratushna, V., Sirghie-colleoni, M., James, M., & Myers, A. (2004). Molecular characterization demonstrates that the *Zea mays* gene *sugary2* codes for the starch synthase isoform SSIIa. *An International Journal on Molecular Biology, Molecular Genetics and Biochemistry*, *54*(6), 865–879.

Zhao, Y., Hasjim, J., Li, L., Jane, J.-L., Hendrich, S., & Birt, D. F. (2011). Inhibition of azoxymethane-induced preneoplastic lesions in the rat colon by a cooked stearic acid complexed high-amylose cornstarch. *Journal of Agricultural and Food Chemistry*, *59*(17), 9700–9708.

Zhu, L., Gu, M., Meng, X., Cheung, S. C. K., Yu, H., Huang, J., . . . Liu, Q. (2012). High-amylose rice improves indices of animal health in normal and diabetic rats. *Plant Biotechnology Journal*, *10*(3), 353–362.

Zhu, L., Liu, Q.-Q., Wilson, J. D., Gu, M.-H., & Shi, Y.-C. (2011). Digestibility and physicochemical properties of rice (*Oryza sativa* L.) flours and starches differing in amylose content. *Carbohydrate Polymers*, *86*(4), 1751–1759.

Zobel, H. F. (1988). Molecules to granules: A comprehensive starch review. *Starch-Stärke*, *40*(2), 44–50.