Resistant Starch and Slowly Digestible Starch

Bilal Ahmad Ashwar, Adil Gani, Asima Shah, Mudasir Ahmad, Asir Gani, Faiza Jhan, and Nairah Noor

Introduction

In human nutrition, starch plays an important role in supplying metabolic energy, which enables the body to perform its functions. Based on the rate and extent of digestion, starch has been classified into different fractions viz. Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS) (Englyst et al. 1992). Starch has been quantified into these fractions using the in vitro Englyst assay: the starch fraction digested within 20 min of incubation has been classified as RDS, the starch fraction digested between 20 and 120 min corresponds to SDS, and the remaining fraction that was not further digested has been classified as RS. RDS induces a rapid increase in the blood glucose and insulin levels, which may cause a series of health complications, such as diabetes and cardiovascular diseases (CVD). SDS is slowly digested in the small intestine, thereby resulting in a slow and prolonged release of glucose into the blood, coupled to the low glycemic response. Thus SDS can be helpful in controlling and preventing hyperglycemia-related diseases. Resistant Starch (RS) is the fraction of starch that is resistant to hydrolysis by α-amylase and pullulanase enzymes in vitro and may be fermented in the colon (Englyst et al. 1982). It is that fraction of starch, which escapes digestion in the GI tract but may be fermented in the colon (Englyst et al. 1996). The end-products of fermentation are carbon dioxide, hydrogen, methane and short chain fatty acids (SCFAs).

RS is measured as the difference between total starch (TS) and the sum of rapidly digestible starch (RDS) and slowly digestible starch (SDS) (Sajilata et al. 2006).

$$\mathrm{RS} = \mathrm{TS} - \left(\mathrm{RDS} + \mathrm{SDS}\right)$$

B. A. Ashwar · A. Gani (✉) · A. Shah · M. Ahmad · A. Gani · F. Jhan · N. Noor
Department of Food Science and Technology, University of Kashmir, Srinagar, India

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RS may escape digestion due to various reasons. The compact molecular structure of starch may limit the accessibility of hydrolytic enzymes (Haralampu 2000). The starch may be physically inaccessible to the hydrolytic enzymes as in grains, seeds and tubers. The starch granules may also be configured in such a manner which prevents their digestion e.g., unripe bananas, raw potatoes, and high amylose maize starch (Nugent 2005). When gelatinized starch is cooled retrograded starch (starch crystals) is formed which is resistant to digestive enzymes. This form of 'retrograded' starch is found in foods like corn flakes, and cooked and cooled potatoes (approximately 5%) (Haralampu 2000). Chemical modifications like esterification, etherification and cross linking of starch also makes it resistant to enzymatic hydrolysis (Lunn and Buttriss 2007). RS has been further classified into five types: RS1, RS2, RS3, RS4 and RS5.

RS1

This form of RS is physically inaccessible to hydrolytic enzymes because it is entrapped within the food matrix such as partly milled grains and seeds. It can be used in a variety of conventional foods as it is stable to most cooking operations (Sajilata et al. 2006).

RS2

The RS2 comprises of native, uncooked granules like raw potato or banana starches, whose crystallinity makes them resistant to enzymatic hydrolysis (Hernandez et al. 2008). These ungelatinized starches are resistant to digestion because of their compact structure (Sajilata et al. 2006). A particular type of RS2 called high amylose maize starch (HAM) is unique because it is stable to most cooking operations (Wepner et al. 1999).

RS3

RS3 is generally called retrograded starch (Wepner et al. 1999). It is formed when starch is first gelatinized and then cooled for retrogradation. During retrogradation the polymer chains reassociate by the formation of inter chain hydrogen bonds to form double helices. The double helices are left handed, parallel stranded and one turn of double helix is 20.8 Å. Retrograded starch has type A crystalline structure (Eerlingen et al. 1993a, b). RS3 content is also affected by degree of polymerization (DP) of amylose, with increase of DP, RS3 content increases, reaching maximum at

100 DP and then remains constant (Eerlingen et al. 1993a, b). DP level of 10–100 is necessary for the formation of double helix (Gidley et al. 1995).

RS4

RS4 is chemically modified starch and include modifications like esterification, acetylation, etherification, phosphorylation or crosslinking. RS4 is further grouped into subcategories based on their solubility in water and the experimental protocols used for analysis (Nugent 2005). Chemical modifications of starch prevent its digestion by blocking the access to hydrolytic enzymes and by the formation of atypical links (Sajilata et al. 2006; Kim et al. 2008).

RS5

Besides structural characteristics, other factors intrinsic to starch rich foods can affect enzyme activity and hence starch hydrolysis. These factors include the amylose-lipid complexes, the presence of native α-amylase inhibitors in starchy foods and non-starch polysaccharides. RS5 is formed by the complexation of amylose with lipids (Fuentes-Zaragoza et al. 2011).

Sources

Naturally cereals, seeds and other starch rich foods are excellent sources of SDS and RS (Charalampopoulos et al. 2002; Gani et al. 2020). Among non processed foods, unripe bananas are the richest source of RS (47–57%). Unripe banana flour was prepared with 17.5% RS, 73.4% total starch and 14.5% dietary fibre (Rodriguez et al. 2008). Tuber starch like potato shows B-type crystallinity which is highly resistant to enzymatic hydrolysis. Raw potato starch contains almost 75% RS (Bednar et al. 2001). Whole grains are rich sources of RS, dietary fibre and oligosaccharides while as flour group contains low concentrations of RS (Slavin 2004). Flour contains two principal components viz. protein and starch, where as whole cereal grain contains the pericarp, aleurone layers and germ that provides the lipids and fibre. Processing of cereal grains alters their chemical composition. The RS content of whole cereal grains was found to be five times higher than their respective flours (Bednar et al. 2001).

Pulse grains contain high contents of RS (Rochfort and Panozzo 2007). RS and total dietary fibre contents of 24.7% and 36.5%, respectively were found in legumes. Various factors are responsible for higher contents of RS in legumes. C-type pattern of crystallinity found in leguminous starches makes them more resistant to

hydrolysis as compared to cereals which have A-type crystallinity (Mir et al. 2013). Leguminous starch contains higher levels of amylose as compared to cereal and pseudocereal starches which reflects their high RS content (Mikulíkova et al. 2008). Quick retrogradation of cooked leguminous starch makes it exceptionally resistant to hydrolysis (Tharanathan and Mahadevamma 2003).

RS content varies from a few percent to 80% in the legumes. It may further increase or decrease due to hydrothermal processing, depending on the variety of legumes and parameters of processing (Giczewska and Borowska 2003).

Processing had a great impact on the RS and SDS content of foods, and generally RS contents are reduced by severe and longer periods of processing. RS content of cooked rice reduced from 12% to 5% during grinding where as RS content of oats reduced from 16% to 3% during cooking (Muir and O'Dea 1992). Autoclaving, baking, flaking, and parboiling are known to influence starch digestibility and the yield of SDS (Brand et al. 1985; Holm et al. 1985; Casiraghi et al. 1993; Kingman and Englyst 1994). During pullulanase debranching and cooling treatment of the cooked waxy maize starch, short-term retrogradation occurs as a result of the crystallization of the amylose fraction, leading to maximum SDS formation (Miao et al. 2009).

Formation of Resistant Starch and Slowly Digestible Starch

Different modifications of starch like physical, chemical, enzymatic treatments, irradiation and genetic modifications have been employed for the formation of RS and SDS. Some of these modifications are described as follows:

Physical Modifications

Physical treatments for preparation of SDS and RS include hydrothermal treatments, recrystallization, polymer-entrapment, and extrusion. When starch is heated to various levels, it leads to the formation of RS and SDS. RS was obtained by cooking the starch above its gelatinization temperature and drying simultaneously on heated rolls like drum driers and extruders (Holm et al. 1988). Gelatinization of starch at 120 °C for 20 min, followed by cooling to room temperature also provides good yields of RS (Garcia-Alonso et al. 1999). High yields of SDS (39.3–56.7%) were obtained by dual retrogradation treatment (gelatinization-retrogradation-gelatinization-retrogradation) in rice starch (Tian et al. 2013). Good yields of RS3 were also obtained by various combinations of time and temperature treatments to various sources of native starch. The temperature treatments were autoclaving the starch at 110 °C (Berry 1986), at 121 °C (Sievert and Wursch 1993), at 127 °C (Berry 1986), at 134 °C (Berry 1986), or at 148 °C Sievert and Pomeranz 1989) for periods ranging from 30 min to 1 h. Recently effects of autoclaving temperature

(140–145 °C) and storing time (24, 48 and 72 h) on resistant starch (RS) formation from high amylose corn starch were investigated. High autoclaving temperature (145 °C) and long storage time (72 h) increased the yield of RS (Dundar and Gocmen 2013). Partial acid hydrolysis (PAH) of the high-amylose corn starch can be used to produce granular RS, which is stable to further heat treatment at atmospheric pressure (Brumovsky and Thompson 2001; Ozturk et al. 2011). PAH followed by heat moisture treatment increased the yield of boiling-stable granular RS to the maximum of 63.2%. Pyrodextrinization has been identified as a way of producing RS which is water-soluble and has non-starch linkages (Laurentin and Edwards 2004). Modification of dry starch through heat treatments, with or without addition of acids is referred to as Pyroconversion. The acids include hydrochloric acid at 0.15% (based on starch dry weight) and orthophosphoric or sulfuric acids at 0.17% (Wurzburg 1995). Pyrodextrins are commercially produced by heating dry, acidified starch in a reactor with agitation. During pyroconversion hydrolysis and transglycosidation of starch occurs which can be facilitated by spraying acid on the starch. A wide range of products that vary in available starch, digestibility, cold-water solubility, swelling power, viscosity, color, and stability were produced during pyroconversion (Ohkuma and Wakabayashi 2001).

Shin et al. (2005) reported that when granular sweet potato starch (50% moisture) is heated to 55 °C, the amount of SDS increases by 200%. It has been reported that hydrothermal treatment of granular sweet potato starch alters its structure from C_b type to A-type as a result of the melting of starch crystallites and subsequent recrystallization. This structure change converts a fraction of amorphous amylose molecules into the crystalline form, thereby decreasing enzyme susceptibility. Miao et al. (2009) showed that controlled retrogradation of partially debranched waxy maize starch can be used to make SDS and RS, which occurs due to the formation of imperfect, low-density B-type crystallites (Miao et al. 2009). Controlled debranching of waxy starch results in the formation of great number of short chains of amylose that are available for chain re-alignment, cross-linking and double helix formation, which leads to the formation of more SDS and RS contents. Other studies have shown that retrogradation correlates with the SDS and RS content of mutant maize; this maize has a higher proportion of long amylopectin chains and linear branch chains of amylopectin with *DP* 9–30. This type of amylopectin probably acts as an anchor point to slow the digestion of branched-chain fractions with $DP > 30$, which as physical entities are the primary constituents of SDS and RS (Zhang et al. 2008). Entrapment or encapsulation of the starch in the structured protein network can be used as a novel method for development of RS and SDS. Starch-encapsulated spheres with 44% SDS were prepared by dropping a homogeneous mixture of 1% sodium alginate (w/w) and 5 g of starch into a 2% $CaCl_2$ solution (w/v) (Hamaker et al. (2007). An SDS product has been generated by using partially gelatinized or plasticized materials to form a low-swelling network of mixed crystallites that consisted of short-chain amylose ($DP < 300$) and basic starch. This network has been formed through cooking or mixing processes, especially extrusion (Innereber and Mueller 2005). In addition SDS has been generated in feed by adding a reducing carbohydrate to comminuted cereal grain, heating the

mixture followed by drying (Winowiski et al. 2005). In other words, physical modifications of the starch that affects enzyme binding and the rate of digestion can be used to modulate starch digestibility for formation of SDS and RS.

Enzyme Treatment

Controlled enzymatic treatment of starch with α-amylase, β-amylase, isoamylase, pullulanase and transglucosidase is an alternative approach to change the chain-length of starch supramolecular structure in order to achieve appropriate digestibility and glycemic response (Shah et al. 2018). RS has been prepared from high amylose starch by gelatinization followed by treatment of slurry with debranching enzymes like pullulanase and isolating the starch product by drying/extrusion (Haralampu and Gross 1998). RS products having at least 50% RS content were manufactured by forming a water-starch suspension, heating the suspension in an autoclave at 100 °C so that full starch gelatinization takes place and then cooling to allow retrogradation of amylose. The best results were obtained at a temperature of 134 °C, with four heating-cooling cycles and a starch: water ratio of 1:3.5 (Pomeranz and Sievert 1990). RS was also prepared by gelatinizing the starch (common corn starch and waxy maize starch), followed by treatment with a debranching enzyme, isoamylase or pullulanase and precipitation of the debranched starch. For precipitation, the suspension was allowed to cool at room temperature, which reduced the solubility of the starch and then the precipitate was heated at 70 °C to dissolve a small portion of the precipitate. Reprecipitation was then employed by cooling of the suspension. This repetition of the dissolving and precipitation processes improved the temperature stability of the resulting aqueous dispersion (Harris and Little 1995). Increased yields of RS were obtained by subjecting the starch to enzymatic hydrolysis (pullulanase, 40 U/g/10 h), autoclaving (121 °C/30 min), storing under refrigeration (4 °C/24 h), and lyophilizing (Reddy et al. 2013).

SDS has been prepared by debranching starch using pullulanase or isoamylase (Shi et al. 2003). In the case of waxy starches, shorter debranching time and high concentrations of debranching enzymes are more suitable for debranching starch to form SDS (Guraya et al. 2001b). A low GI maize starch with some branched structure has been developed by partial α-amylase treatment and retrogradation, and the slow digestibility was retained even after cooking (Han et al. 2006). Shorter chains of amylopectin and noncrystalline amylose molecules were rapidly digested, while DP_n 121 chains showed the greatest resistance to digestion, followed by DP_n 46 chains. A similar trend was reported in the formation of SDS from commercial starch by controlling the hydrolysis of gelatinized starch with α-amylase (Hamaker and Han et al. 2006). A novel slowly digestible storage carbohydrate comprising of more than 90% amylopectin was produced by treating a native root or tuber starch with a branching enzyme derived from a microorganism with a branching degree of at least 8.5–9% (Vander-Maarel et al. 2008). Moreover, it was reported that both the increase in branch density and the crystalline structure of starch enhances its slow

digestion property through the partial shortening of amylopectin A and B1 exterior chains, as well as linear chains of amylose, through the action of β-amylase and maltogenic α-amylase (Ao et al. 2007). This correlated closely with an increase in the number of α-1, 6 linkages and a simultaneous decrease in the number of α-1, 4 linkages. The enzyme-treated starch consists of B- and V-type crystalline structures, which increases the resistance of starch to digestion. These studies suggested that enzymatic debranching of the exterior chains of amylopectin molecule can change its structure and form higher proportions of SDS and RS.

Chemical Modifications

In many processes, starch is being modified by chemical reagents to improve functionality and create commercially valuable, starch based products. The most common chemical treatments are acid treatment, oxidation, cross-linking and substitution including esterification and etherification. Recently studies have focused on such treatments in SDS and RS production (Zhao et al. 2012; Ashwar et al. 2016; Ashwar et al. 2018). The enzymatic resistance in RS4 is done by cross linking with chemical agents (Haynes et al. 2000). Cross linked starches are obtained by reaction of starch with bi- or polyfunctional reagents like phosphorus oxychloride, sodium trimetaphosphate, or mixed anhydrides of acetic acid and dicarboxylic acids like adipic acid. Cross-linking of rice, wheat, corn, potato, tapioca, oat and mung bean starches using sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin or phosphoryl chloride ($POCl_3$), produced type 4 resistant starch (Seib and Woo 1999; Zhao et al. 2012). These authors explained that the levels of RS in wheat starch cross-linked with 2% $POCl_3$, 12% STMP/STPP, and 2% epichlorohydrin were 85.6, 75.6 and 75.8 g/100 g starch, respectively. Sang et al. (2010) prepared phosphorylated wheat starch with high levels of the RS (68.7%) and SDS (24.4%). Cross-linking when carried out by sulphonate and phosphate groups between starch molecules through their hydroxyl groups brings resistance to enzymatic hydrolysis (Hamilton and Paschall 1967). Cross-linking of starch with mixtures of STMP and STPP under alkaline conditions restricts swelling of starch and imparts increasing resistance to digestive enzymes (Woo and Seib 2002). Simsek et al. (2012) prepared acetylated bean starch with high levels of the RS (44%). Acetylation of starch increased the RS content in bean, as a result of acetyl groups which blocks the action of digestive enzymes (Chung et al. 2008). Modification of starch with octenyl succinic anhydride is known to increase levels of SDS and RS more than other modifications such as acetylation, hydroxypropylation, or crosslinking (Han and BeMiller 2007; Juansang et al. 2012). Esterification with octenyl succinic anhydride (OSA) has been shown to be the most potent method of modifying waxy starch to form SDS, followed by combined modifications like crosslinking-hydroxypropylation, acetylation and crosslinking (Han and BeMiller 2007). The modified starch with attached OSA molecules may act as uncompetitive inhibitors to reduce the enzyme activity and thereby cause slow digestion of starch. As these

studies showed, chemical modifications of starch can be used to prepare SDS and RS, but clinical and toxicological trials needs to be performed in order to evaluate the safety and efficacy of SDS and RS consumption.

Genetic Modification

Genetic modification of starch biosynthesis has been used to develop a strategy for generating new cultivars with desired functionality through extensive breeding and characterization of the resulting cultivars. Genetically controlled factors that affect the starch functionality include structure of starch, content of starch, interaction of cell components, and starch granule architecture. Waxy starches may be more suitable for developing SDS, since their fine amylopectin structure (i.e., the distribution of branches and chain length) is more critical for SDS formation (Guraya et al. 2001b). In one study, SDS (long-chain amylopectin starch) was developed from maize by over expressing a particular enzyme involved in starch biosynthesis (Moallic et al. 2006). This starch, with high granule crystallinity, has few short chains of amylopectin and more intermediate and long chains. Other study showed that genetic mutants containing amylopectin molecules with either a high proportion of short chains with $DP < 13$ (particularly A chains with DP 5–9) or a high proportion of long chains with $DP \geq 13$ (particularly intermediate to long B chains with $DP > 30$) contain greater proportions of SDS than wild type (Zhang et al. 2008). According to a study by Benmoussa et al. (2007), development of SDS and RS is positively correlated with the presence of both long and intermediate/short chains, respectively, while it is negatively correlated with the lowest proportion of extremely short chains. They also found that the channels in starch granules can regulate starch digestibility, since starch granules with channels are digested from the interior, and more extensive channelization of starch gives more access to the hydrolytic enzymes (Benmoussa et al. 2006). Therefore, genetic modification has the potential to produce ideal starch with high contents of SDS and RS.

Factors Influencing the Formation of RS and SDS

Various factors have been reported to influence the formation of RS and SDS. These are described as:

Starch Structure

Starch is semicrystalline in nature synthesized mostly as spherical granules in plant tissues. These granules are composed of alternating concentric layers of ordered crystalline and less-ordered amorphous lamellae extending from hilum to the surface

of granules. The crystalline lamellae are formed from the amylopectin short branch chains arranged in clusters; these crystalline lamellae are interspersed with amorphous lamellae that consist of branching points of amylopectin and amylose molecules. The sub-chains of the amylopectin has been classified into three types. A chains (outer chains) are the shortest among the three chains (CL 6–15) and are α-(1, 6)-linked to B chains. The B chains are linked in the same way and bear one or more A chains and/or B chains. Depending on their respective length and the number of clusters they span, B chains are further classified into B1, B2, B3 and B4 chains (with one to four clusters). B1 and B2 chains have CL of 15–25 and 40–50, respectively, with B3 and B4 chains being much longer. The single C chain per amylopectin molecule contains the sole terminal reducing group and carries other chains (Donald 2004). Within this structure, the linear chains lie in the region of high molecular order, and the branch-points lie in the region of low molecular order. These linear chains can form double helices to make up the crystalline structure. On the basis of wide-angle X-ray diffraction scattering studies, native starch has been classified into four types viz. A, B, C and V. The A type is characteristic of most cereal starches, while the B type is characteristic of potato starch, other root starches, amylomaize starch, and retrograded starch. The C type is a combination of A and B types, and is found in smooth pea and various bean starches. The V type can be found only in starch after gelatinization and the formation of amylose helical complexes with lipids or related compounds.

Studies of X-ray diffraction of RS showed that chain fragments were packed in a B type crystalline structure with enlarged crystal lattice which contributes to the formation of RS. Any treatment that eliminates starch crystallinity (e.g., gelatinization) or damages the integrity of the plant cell or tissue structure (e.g., milling) increases access to enzymes and reduces the RS content, whereas recrystallization and chemical modifications increases the RS content (Englyst and Cummings 1986; Adebowale et al. 2009; Kim and White 2013). Further high amylose content of starch is known to lower starch digestibility (Chung et al. 2009). High amylose maize starches with very long chains might be perfectly ordered into double helices to form resistant starch (Ozturk et al. 2011). Higher contents of resistant starch were found in Hylon VII than in Hylon V (high-amylose genetically modified corn starches) which might be because of higher amylose content in Hylon VII (Dimantov et al. 2004). Margareta Leeman et al. (2006) claimed that high amylose starch resists enzymatic digestion due to its internal structure and B-type crystallinity. Native cereal starch has been classified as an ideal SDS, since its structure makes it to be digested slowly (Zhang et al. 2006a, b). They found that the A-type semicrystalline structure of native cereal starch, including the distribution of perfect crystalline regions in both crystalline and amorphous lamellae explains this slow digestion property. The high proportion of SDS in cereal starch was also correlated with higher proportion of short A chains with *DP* 5–10. The mechanism of slow digestion property of native cereal starch involves enzymatic digestion from inside out and layer-by-layer. Enzymatic digestion begins in interior channels and at surface pores, and then side-by-side digestion gradually enlarges the channel by simultaneously hydrolyzing crystalline and amorphous regions. Native starch is hydrolysed

more slowly than gelatinized starch; since gelatinization has lost the crystalline structure of starch, allowing greater access to enzymes without the obstructions caused by α-glucan associations, such as double helices or by amylose-lipid complexes in cereal starches (Tester et al. 2002). Other studies claimed that the dispersed, amylopectin fine structures with high branch density, either long or short internal chains as well as short terminal non reducing ends, leads to the slow digestion property, because of the inherent structure of amylopectin molecules (Hamaker et al. 2007; Zhang et al. 2008). The plasma glucose response after consuming raw maize starch was slow and sustained, which is characteristic of SDS (Seal et al. 2003). The structure of SDS is composed of imperfect crystallites and amylopectin with a high branching pattern and density, and this is most likely the cause of slow digestion property.

Heat and Moisture

Heat and moisture content are important factors for development of SDS and RS. When native starch is heated in excess water, the starch granules undergo gelatinization. The extent of gelatinization depends on the temperature, time, water content and degree of shear during the process. As described previously, native starch (A-type) is an ideal SDS and the slow digestibility changes during cooking or processing. Incomplete gelatinization can be achieved by lowering the temperature, decreasing the moisture content, or shortening the heating time. In this way, low GI benefits of SDS and RS may be retained. When partially gelatinized waxy rice starch was heated at different temperatures (60, 65, or 70 °C) for 5 min, they showed different digestibility rates after retrogradation (Chung et al. 2006). The amounts of SDS and RS positively correlated with the relative enthalpy of the partially gelatinized starches. In cereal products, such as parboiled rice, barley porridges, biscuits and pasta, the degree of gelatinization or limited swelling of starch, which is determined mainly by the cooking time and temperature, moisture level, largely influences the formation of SDS and RS (Wolever et al. 1986a, b; Holm et al. 1992; Granfeldt et al. 1994; Garsetti et al. 2005). Heat-moisture treatment usually refers to the incubation of starch at low moisture content (<35% w/w) for a certain period of time at a temperature below the gelatinization temperature, but above the glass transition temperature, while as annealing is performed in excess water or at an intermediate water level (≥40% w/w) (Jacobs and Delcour 1998; Tester and Debon 2000). Heat–moisture treatment does not destroy structure of starch granules, but it alters its crystalline packing; for example, the B type of starch can be converted to the A or C type, whereas the annealing technique can modify the binding forces between the crystalline and the amorphous matrix (Stute 1992). Therefore, hydrothermal treatment can be used as a method to form SDS and RS. Anderson et al. (2002) adjusted both nonwaxy and waxy rice starches to 20% moisture, after that heated them to their melting temperature (*T*m) in a differential scanning calorimeter (DSC), and held them there for 60 min. They observed that these starches were

more slowly digested than unheated samples. Severijnen et al. (2007) used these principles to study the production of a sterilized liquid product with a low GI. When the modified high amylose starch was heated above 120 °C for 4–5 min., the SDS content increased and reached a maximum, where it remains stable for several months when stored at 4 °C. According to Woortman and Steeneken (2004), high SDS content was achieved when a starch product with an amylose content of below 50% was heated to at least 170 °C under mild acidic conditions, followed by rapid cooling. High amylose starch is rich source of RS2 (Berry 1986), which after heating and cooling gives RS3 in high yields (Sievert and Pomeranz 1989) or retrograded starch (Englyst et al. 1992). Retrograded amylose in wheat, maize, peas and potatoes was found to be highly resistant to digestion (Ring et al. 1988). Park et al. (2009) reported that temperature cycled storage increased the formation of resistant starch and reduced the GI (glycemic index) of waxy corn starch. Borczak et al. (2014) claimed that prolonged frozen storage of wheat-flour rolls significantly increased RS formation. Dual-retrogradation treatment was more efficient as compared to single retrogradation (Tian et al. 2013). Repeated autoclaving of wheat starch increased the RS upto 10%. Retrogradation of amylose was recognized as the main factor for the formation of RS and higher amounts were obtained with repeated autoclaving (Dundar and Gocmen 2013; Bjorck et al. 1990). On storage, gelatinized starch pastes undergo retrogradation to semicrystalline structure that resists enzymatic digestion. Wheat bread and corn flakes are rich sources of this type of RS where as cooked and cooled potatoes have only 25% of RS3 (retrograded starch) (Englyst and Cummings 1985).

Interactions of Starch with Other Components

Interactions of starch with other food components are known to influence the formation of SDS or RS. Two important types of starch interaction with other components involve formation of starch-lipid complexes and starch-protein interactions. Interaction of starch with the protein is thought to reduce the rate of α-amylolysis of starch in cereal and legume products (Wursch et al. 1986; Jenkins et al. 1987; Colonna et al. 1990; Biliaderis 1991). According to Granfeldt and Bjorck (1991), a dense and viscoelastic gluten network surrounds the starch granules in pasta products which restricts the swelling and leaching of starch molecules during boiling and also reduces the access of enzymes to the starch. The interaction of starch with protein also limited the glycemic response of starch in white bread made from regular flour, while gluten-free bread showed a higher glycemic and insulinemic response (Jenkins et al. 1987). In another study, mixture of potato starch and albumin protein was autoclaved and then cooled to −20 °C, and effect of albumin on digestibility of potato starch was studied. It was found that added albumin reduced the content of resistant starch (Escarpa et al. 1997). In a study by Holm et al. (1983), it was claimed that amylose molecules formed complexes with lysolecithin, and these complexes were degraded slowly and were completely absorbed in the GI tract of rats within

120 min. As a result, the amylose complex produces lower plasma glucose and liver glycogen levels than does free amylose. Murray et al. (1998) evaluated apparent digestibility of starch in ileal-cannulated dogs that were fed enteral diets containing debranched amylopectin-lipid V-complex or RS. They found that the ileal and total GI tract digestibilities of the control, V-complex, and RS diets were 89%, 76%, and 43%, respectively, which indicated that the diet containing V-complex starch lowered the carbohydrate digestibility and hence the serum glucose and insulin responses. Differential scanning calorimetry (DSC) was used to study the effect of sodium stearoyl lactylate (SSL), lysophosphatidyl choline (LPC), and hydroxylated lecithin (OHL) on autoclaved amylomaize starch (Czuchajowska et al. 1991). Differential scanning calorimetry (DSC) peaks at around 95–110 °C indicated the formation of complex compounds between amylose chains and lipid, and the peak at about 155 °C indicated the presence of resistant starch (RS). However lower yields of RS were observed from lipid complexed samples as compared to autoclaved and cooled control when subjected to amylolysis by thermostable bacterial α-amylase and amyloglucosidase. Amylose recrystallization which is important in resistant starch formation is adversely affected by complexation of amylose with LPC and SSL. In another study, influence of endogenous lipids on wheat starch showed that defatting of the starch samples resulted in decrease of the RS content. On addition of SDS to defatted wheat or amylomaize starch, resistant starch yields decreased significantly. X-ray diffraction and DSC techniques confirmed formation of amylose-lipid complexes in the presence of both endogenous lipids as well as added lipids (SDS) (Eerlingen et al. 1994).

In addition to the interaction of starch with lipids and proteins, it has also been found that starch can interact with soluble fibers (β-glucans, guar gum, psyllium, or pectin), antinutrients (enzyme inhibitors, phytates, tannins, lectins or saponins), organic acids and sugars (Biliaderis 1991; Bjorck et al. 2000; Pi-Sunyer 2002). Enzyme inhibitors like phytic acid, polyphenols, and lectins present in leguminous seeds, have been found to inhibit in vitro digestion and hence the glycemic index of starch (Thompson and Yoon 1984). Both amylases and intestinal maltase activity are inhibited by tannic acid (Bjorck et al. 1987). Since phytic acid inhibits the amylolysis, an increase in phytate content decreases starch digestibility (Thompson and Yoon 1984). Brennan et al. (1996) reported that the rate of starch hydrolysis slowed down significantly when the starch granules and surrounding bread matrix were coated with a layer of galactomannan mucilage, which acted as a physical barrier to enzyme-starch interactions and hence the release of hydrolyzed products. Guar gum has been found to increase the viscosity of digesta and reduce the rise in postprandial glycemic response that occurs due to the reduction in rate of gastric emptying. Starch blockers (α- amylase inhibitors) inhibits in vitro α-amylase activity or binds to starch substrate, indicating that these have the potential to interfere with the digestion of starch in vivo and hence modulate the glycemic effect of SDS and RS (Giri and Kachole 1998; Obiro et al. 2008). Potato starch gels showed decreased yield of resistant starch in the presence of ions like calcium and potassium (Escarpa et al. 1997) which may be reflected to the prevention of hydrogen bond formation between amylopectin and amylose chains.

Processing Conditions

Processing/cooking, post processing and storage conditions (retrogradation) influence the formation of SDS and RS in food. This fact is of great concern for the food industry, since it offers the possibility of increasing the SDS and RS contents of processed foods and foodstuffs. Autoclaving, pressure-cooking, baking, flaking and parboiling, among other methods, are known to influence the starch digestibility and the yield of RS and SDS (Brand et al. 1985; Holm et al. 1985; Casiraghi et al. 1993; Kingman and Englyst 1994; Ashwar et al. 2016; Ashwar et al. 2017). Holm et al. (1985) demonstrated that starch in flaked whole grained wheat was more resistant to digestion than that in boiled, steam-cooked and popped wheat during in vitro assay. In a study of Casiraghi et al. (1993), both parboiled and quick cooking parboiled rice were digested more slowly with a lower GI than polished rice, which was related to the availability of starch for α-amylase. Autoclaving of red kidney beans has been shown to increase the blood glucose and insulin response as compared to boiling at atmospheric pressure, and this may be due to thermal or mechanical alteration of the structure of seeds and may also be related to the release of physically inaccessible starch due to mechanical disruption of cell walls (Tovar et al. 1992). Granfeldt et al. (2000) reported that thick rolled oats cause lower glycemic responses than thin flakes or reference bread. Boiling and pressure-cooking significantly decreased the SDS content in three *Doongara*, *Inga* and *Japonica* varieties of rice and the amylose content affected starch digestibility, which has been attributed to the retrogradation process (Sagum and Arcot 2000). According to Guraya et al. (2001a), when 10% waxy and nonwaxy starch suspensions were debranched with pullulanase, followed by heating and cooling treatments to allow the crystallization or gelling to occur, the digestibility decreased because of the formation of crystalline structures or double helices. During pullulanase debranching and retrogradation treatments of the cooked waxy maize starch suspensions, short-term retrogradation have occurred as a result of gelation and crystallization of amylose molecules, leading to the formation of SDS; in contrast, long-term retrogradation occurs during storage of starch gels due to the amylopectin molecules (Miao et al. 2009).

Determination of Resistant Starch and Slowly Digestible Starch

Different methods have been developed for the determination of RS with significant differences in sample preparation, enzymes used, and the establishment of experimental conditions that mimic gastrointestinal environment. Most of the methods are employed for the determination of total RS, but some specific methods have been developed for the quantification of RS1, RS2 and RS3.

Basic method for the determination of RS was proposed by Englyst et al. (1982). Briefly, in this method 100–200 mg sample is mixed with sodium acetate buffer at pH 5.4 and heated for 1 h at 100 °C to gelatinize the starch. An enzyme mixture (α-amylase, pullulanase and amyloglucosidase) is added and hydrolysis is carried out at 40 °C for 16 h. Absolute ethanol is added to precipitate nonhydrolyzed starch and to terminate enzymatic activity. The pellet is collected by centrifugation and washed with 80% ethanol twice. The residue is dried with acetone and then treated with 2 M KOH for 30 min at room temperature to solubilize the starch. An aliquot of alkali digest is mixed with 2 M acetic acid and amyloglucosidase and then incubated at 65 °C for 1 h. After cooling and centrifugation, neutral sugars (glucose, mannose, galactose, arabinose and xylose) in the supernatant are analyzed by GLC (gas–liquid chromatography). The amount of glucose detected by GLC represents the amount of resistant starch (RS3) in the sample.

The above procedure was later modified by Englyst et al. (1992) to better mimic human gastrointestinal conditions. The authors proposed that enzymatic hydrolysis can be carried out at 37 °C instead of at 40 °C. Also significant reduction in the duration of enzymatic digestion is used. Instead of 16 h of sample digestion with enzymes, the protocol uses sequential removal of aliquots of enzyme digest at 20 min and at 120 min from the beginning of the digestion. The rationale to terminate enzymatic hydrolysis at 120 min is that the release of glucose reached a plateau at this time interval. The starch fraction digested within 20 min of incubation is classified as RDS, the starch fraction digested between 20 and 120 min is classified as SDS, and the remaining fraction that is not further digested is RS.

Goni et al. (1996) further modified the procedure by decreasing the sample pH to 1.5 with HCl–KCl buffer to simulate gastric pH. Hydrolysis with pepsin at 40 °C for 1 h is carried out. To simulate conditions in the small intestine sample pH is adjusted to 6.9 with tris–maleate buffer. Instead of using a group of enzymes, hydrolysis is carried out with α-amylase only for 16 h at 37 °C. Glucose content is determined by the colorimetric method.

The Megazyme assay kit is widely used in analytical laboratories for the determination of RS and is the basis of both AACC Method 32–40 and AOAC method 2002.02 (Megazyme 2008). In this method samples are ground to a coarse meal which can pass a 1-mm sieve. Initial boiling of samples in acetate buffer and the use of pullulanase is eliminated. Instead a mixture of α-amylase and amyloglucosidase is employed to hydrolyze starch in raw or processed food samples. Hydrolysis is carried out at 37 °C for 16 h. The incubation time with amyloglucosidase is 30 min at 50 °C. This method uses the glucose oxidase-peroxidase colorimetric assay (GOPOD) to determine glucose concentration in the final hydrolysate. The Megazyme protocol for RS determination is not applicable to the determination of SDS and RDS.

Other modifications have been made to the procedure of RS determination particularly in the sample preparation step with the intention of simulating the in vivo digestion. However it is obvious that significantly different levels of RS would be detected in similar foods because of wide variations in analytical protocals, including differences in the enzymes used and in their activity, concentration and sequence of application, and dissimilarities in the conditions of experimental protocals.

Conclusion

Epidemiological studies suggest that low-GI foods have beneficial metabolic effects like the potential to reduce insulin resistance and improve certain metabolic conditions. However, there are very few commercially available low-GI foods in the market, which fails to meet the growing needs of patients with diabetes, obesity and related disorders. SDS and RS as the novel functional components in products deliver a slow and prolonged release of glucose when ingested, resulting in a lower GI. RS has recently been recognized as a dietary starch that escapes digestion in the small intestine and is fermented in the colon producing short chain fatty acids that provides various health benefits including prevention of colon cancer, reduced risk of diabetes, reduction in total cholesterol, promotion of growth of beneficial microflora of colon, improved laxation etc. Interaction of RS and SDS with certain nutrients like wheat bran, cellulose and protein promotes its health benefits. Technically, it is possible to prepare RS and SDS by different physical, enzymatic and chemical modifications. The unique properties of RS and SDS, that is, bland flavor, fine particle size, high gelatinization temperature, good extrusion and film forming qualities and lower water holding properties make the formulation of wide range of food products possible with improved organoleptic qualities as compared with traditional high-fibre products.

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