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RESISTANT STARCH: DETERMINANTS AFFECTING THE CONTENT AND POTENTIAL BENEFITS FOR IMPAIRED GLUCOSE METABOLISM

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ABSTRACT

The development of type 2 diabetes is associated with modifiable lifestyle factors such as diet. Thus, dietary measures have become one of the cornerstones in the management of type 2 diabetes. Resistant starch (RS) has been proposed as an alternative food source beneficial for people with diabetes as it resists digestion by digestive enzymes. This review aims to compile the findings from studies that evaluated the factors involved in improving RS content in various foods and food products. Several studies have shown that the availability of RS in foods is greatly influenced by certain factors. Among the factors are the amylose and amylopectin content of the starch itself, the processing method, cooking method and storage conditions. Interestingly, several studies were also done to evaluate the inhibitory activity of digestive enzymes in food. The findings from these studies may provide insight into choosing a healthier source of food as well as appropriate preparation methods for starch-based food for health-conscious individuals, especially those with diabetes.

KEYWORDS: Resistant starch, Retrogradation, Glycaemic, Diabetes, Type 2 diabetes

INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disorder in which the body is unable to maintain glucose homeostasis due to two main factors: decreased pancreatic β-cells function and the inability of cells in the body to respond to insulin secretion. The worldwide prevalence of T2D continues to rise, and it is predicted that by 2030 there will be 578 million people living with diabetes (1). Since the development of T2D is associated with modifiable lifestyle factors, e.g., diet (2), dietary measures have become one of the cornerstones in the management of T2D.

Starches are the most widely consumed type of carbohydrate in all parts of the world. They are classified into three types based on their degree of hydrolysis by α-amylase: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Starch with a high glycemic index (GI) has inimical effects on glucose metabolism as it causes a rapid rise in blood glucose which will eventually lead to the progression of insulin resistance (3). However, RS has been proposed as an alternative food source beneficial for people with diabetes as it resists digestion by digestive enzymes.

In general, RS is the fraction of starch that resists digestion by digestive enzymes (α-amylase and α-glucosidase) in the stomach and small intestine which later ferments in the large intestine. As a result, the amount of glucose monomers released and absorbed into the bloodstream will be reduced which leads to a reduction in postprandial insulinemia (4). It has been demonstrated in a study that consumption of RS improves insulin sensitivity in men (5). To date, RS has been classified into 5 subtypes: physically inaccessible starch (RS1), native B-type and C-type polymorphic starch granules (RS2), retrograded starch (RS3), chemically modified starch (RS4) and amylose-lipid complexes (RS5) and this classification was made based on the physical and chemical properties of the starch (6).

Despite the benefits of RS in the dietary management of T2D, the availability of RS in foods is greatly influenced by certain factors. Among the factors are the amylose and amylopectin content of the starch itself, the processing method, cooking method, storage conditions and how it is ingested (4). A large number of studies have been carried out to evaluate the effects of changes in these factors on modifying RS content in food. Hence, this review aims to compile the findings from these studies to understand the roles of these factors in improving RS content in various foods and food products that are suitable as an alternative source of food for people with diabetes.

MATERIALS AND METHODS

Search Strategy

Three databases were searched for original articles (Scopus, ScienceDirect and PubMed) from the year 2011 to 2021 using the term “resistant starch” and “diabetes”. Publications with available abstracts were reviewed and limited to studies published in the English and Malay languages. Only publications on in vitro studies related to the search terms were included. Review articles, letters to Editors and articles with no full article access were excluded. Articles were screened for duplication and eliminated.

RESULTS

A total of 189 articles were found from the three search engines: Scopus (89 articles), Science Direct (15 articles) and PubMed (85 articles). A refined search found 74 articles that were relevant to in vitro studies related to the search terms. Further assessment based on the full text availability, peer-reviewed articles and library collection access resulted in only 33 full articles, which were included in this review.

DISCUSSION

This review has summarised in vitro studies done in the past 10 years on different sources of starch or starch-based food products prepared or processed in different conditions or methods to assess as well as modify their RS content. Since high RS foods tend to have a lower GI value, this may help delay the absorption of glucose into the bloodstream, consequently helping to control blood glucose levels in individuals with T2D. Several attempts have been made to modify and improvise not only the RS content but also the amylose or amylopectin content by performing different cooking styles, food additions, physicochemical, enzymatic modifications or by using genetic alterations. Interestingly, several studies were also done to evaluate the antidiabetic properties of retrograded starch on the gastrointestinal tract by studying the inhibitory activities of α-glucosidase and α-amylase enzymes, which play an important role in inhibiting or delaying carbohydrate digestion and glucose absorption, which in turn prevents the blood glucose spike.

Amylose, amylopectin & resistant starch content

Amylose, an α-(1,4)-linked glucosidic chains, and amylopectin, a highly branched component formed by α-(1,4)-linked units with α-(1,6)-linked branches, are found in different proportions in different sources of starch. The content of these two components greatly affects starch digestibility as they were found to be correlated with the formation of RS. A higher content of amylose and RS in starch may lead to a marked decrease in GI since amylose is digested slower and RS resists hydrolysis by digestive enzymes (7,8,9,10,11). Guzman et al.(12) screened 27 breeding lines of rice grains and selected five rice varieties that represent low, intermediate and high GI rice for further evaluation on amylose and amylopectin content. Amylose and amylopectin content of the five varieties ranged from 0.42 to 7.96% and 4.51 to 13.95%, respectively, and were found to be negatively correlated with the estimated GI. Kumar et al. (13) studied the effect of food combinations on rice starch digestion properties, but first screened 100 rice varieties for their GI, RS and amylose content. In this study, they found that Lalat MAS contained the highest amylose, with a value of 27.36%, while Savitri had the lowest amylose content of 13.42%. As for RS, the highest value (2.93%) was found in Gayatri, while the lowest GI was found in Shaktiman. The amylose content, resistant starch and estimated GI of rice flour from twelve varieties, three from each of five bran colours (white, brown, red, and purple) were reported to vary among different rice bran colours. Amylose content among the three varieties of each pigmented rice flour ranged from 1.20 to 28.90% with the lowest amylose content (1.20%) recorded in one of the purple varieties, while the highest amylose content was recorded in one of the white varieties (28.90%). The RS was the highest (6.13%) in BPT brown rice flour but not significantly different from the three purple brown rice flour samples where the values ranged from 5.33 to 5.69% (14). These findings could be used as baseline information towards making a healthier choice of rice, especially for health-conscious consumers.

Apart from rice, there was also research done in potatoes to screen for cultivars that have a higher content of amylose and RS. In this particular study, 46 Indian potatoes were cooked and analysed for amylose as well as RS content. The amylose content reported for all cultivars ranged from 10.8 to 27.6 mg/100 mg, with the highest value of 27.6 mg/100 mg found in Kufri Chipsona-3. In terms of RS content, values recorded ranged from 1.22 to 1.93 mg/100 mg with Kufri Garima displaying the highest value of 1.93 mg/100 mg (15). Breadfruit (*Artocarpus altilis*), a low-calorie tropical staple crop rich in dietary fibre and protein, is considered good for people with impaired glucose metabolism (16,17). Daley et al. (18) evaluated the RS content of breadfruit-derived flour that was prepared from 21 different cultivars to explore their potential as a special diet for the management of obesity and type 2 diabetes. From the study, it was reported that the RS content of the breadfruit cultivars ranged from 28.16 to 50.53 g/100 g with the Ma'afala' cultivar showing the highest level of RS. A study on amadumbe (*Colocasia esculenta*), commonly known as taro showed similar contents of total starch (approx. 95%) for both wild and cultivated types. The amylose content in wild amadumbe was 20%, which is almost double compared to the cultivated amadumbe (12%). The amount of RS was also slightly higher in the wild amadumbe than in the cultivated amadumbe (19). However, a study on the porridge and injera made out of six different varieties of tef revealed that although all the tef varieties studied were high in apparent amylose content, the estimated GI of the porridge and injera produced were mostly high. The RS content of the porridge and injera produced also decreased notably compared to the flours of the tef varieties (20).

Despite the naturally available options of high amylose food, researchers all around the world spare no effort in developing new cultivars or food products with improved health benefits. In a study on low-glutelin rice cultivars, Guo et al. (21) successfully generated mutant lines with 1.8 fold increase in apparent amylose content (AAC) as well as increased RS content by approximately 6% via site-specific mutations on the starch branching enzyme IIb (SBEIIb) gene using the CRISPR/Cas9 technology. Another novel mutant rice line was developed by introducing the starch synthase IIa (SSIIa) gene and/or granule-bound starch synthase I (GBSSI) gene from an indica rice cultivar into a japonica rice-based BEIIb-deficient mutant line, be2b. This technique managed to produce a higher level of amylopectin chains than those in be2b. In addition, the introduction of the GBSSI gene significantly increased amylose content (by approximately 10%) in the endosperm starch. Indirectly, these new mutant lines will contain the same RS content or slightly higher than that of the be2b parent line (22). Chou 2418, an amylopectin long-chain (ALC) rice developed through cross-fertilisation between EM10 and a high amylose rice cultivar has also been shown to have a significantly higher apparent amylose content of 56.8% when compared to other cultivars (23). Another genetic approach in modifying amylose content was done in durum and bread wheat varieties through targeting induced local lesions in genomes (TILLING). In this method, single nucleotide polymorphisms (SNPs) were induced through chemical mutagenesis to generate mutant alleles of SBEIIa in durum and bread wheat varieties and it resulted in lines with significantly higher amylose and RS contents when compared to their WT counterparts (24). Raja et al. (25) discovered six deleterious variants in the genes through the EcoTILLING approach with the potential to increase RS and decrease the hydrolysis index (HI) of rice starch. It was reported that the accessions with SNP variants in all the three starch synthase (SS) genes were found to have the highest RS content (7.5 to 7.6%), followed by accessions with variants in two SS genes (6.8 to 7.4%) and accessions with SNP variants in a single SS gene recorded the lowest RS content, ranging from 4.1 to 6.1%.

Yang et al. (26) have done a mapping, verification and elucidation of the mutated gene sbe3-rs, for resistant starch in rice, 'Jiangtangdao1' which was known to have high RS and AAC. Measurement of RS content in the F3 population (cross-fertilisation between 'Jiangtangdao1' and Miyang 23) revealed that the RS content within this family ranged from 0.4 to 13.67% with the majority of them having low RS content (0.4-1.0%). This indicated that the mutated RS locus was recessive to regular-low RS. In sequence analysis, they identified a missense mutation in the SBE3 coding region that changed Leu599 to Pro599 in the mutant gene. This sbe3-rs gene was thought to have an impact on the protein conformation of SBE3, hence, it might lead to biosynthesis of high RS in 'Jiangtangdao1'. They later conducted another study to further verify this theory. A gene complementary test was done by introducing the wild-type OsSBEIIb into the sbe3-rs mutant 'Jiangtangdao1'. They discovered that the genetically complemented lines not only had restored seed-related traits but also had significantly lower RS content than 'Jiangtangdao1' and were nearly equal to the wild type. This demonstrated that the wild-type rice SBEIIb gene could complement the mutant sbe3-rs gene in 'Jiangtangdao1' and that the high RS content in 'Jiangtagndao1' was caused by a missense mutation that changed Leu599 to Pro599 (27).

Apart from evaluating nutritious foods for their health benefits towards safe consumption by people with impaired glucose metabolism, studies on developing nutritious and healthy food products out of these nutritious foods have also shown promising results. Song et al. (28) embarked on a study to develop low-sugar cookies as a healthy snacking option by substituting the normally used ingredients such as wheat flour and sucrose with healthier and nutritious ingredients: butter, xylitol and high amylose maize flour (HAMF) with the optimal amounts of 183 g, 150 g, and 341 g; respectively. Results from this study showed that not only did the cookies exhibit higher proportions of long amylose chains, but also higher proportions of long amylopectin chains and lower proportions of short amylopectin chains. RS content of the cookies was also found to be positively correlated with butter, xylitol, and HAMF. Water chestnut, a well-known low GI food was used as a base ingredient in developing healthy muffins and crackers. The addition of barley flour in the preparation has resulted in an increased RS content of the final products compared to the individual RS content of the water chestnut and barley, respectively. However, an increasing pattern in GI values was observed with an increasing portion of barley being incorporated in the preparation of both muffins and crackers. The mixture with a ratio of 70:30 of water chestnut and barley flours was considered optimal, as with this ratio the developed muffins and crackers showed considerably low GI (29,30). Cookies made from the addition of arrowroot (*Maranta arundinaceae*), cinnamon (*Cinnamomum verum*) and porang (*Amorphophallus oncophyllus*) glucomannan has also been shown to be a good alternative snack for health-conscious individuals due to the blood glucose-lowering properties of the added ingredients. Despite having a considerably high level of RS (2.23% per 100 g), the developed cookies also had a low GI (48.2) (31). Hence, using starch with a higher content of amylose as a food source or as an ingredient in developing healthy food products is a better option as it has been proven that this kind of starch yields more RS and has a slower digestion rate, which in turn leads to lower postprandial glycaemic and insulin responses. This may be due to more linear chains of amylose forming a compact structure that reduces binding by digestive enzymes, resulting in a reduced rate of amylolysis. Amylopectin, on the contrary, is easily digested due to its highly branched structure. However, research has shown that when amylopectin is de-branched, the amount of RS increases significantly, implying that linear molecules derived from amylopectin could play a role in retrogradation (32,33,34,35).

Table 1. The RS, amylose and amylopectin content as well as the glycaemic index (GI) of various food and food products subjected to different processing and cooking methods, storage conditions and genetic alterations.

Reference	Formulation/ source/ treatment	Findings			
		RS content	GI	Amylose content	Amylopectin content
Guzman et al. (12)	27 breeding lines of rice grains only five contrasting lines were subjected to detailed analyses (proportions of total starch, RS, amylose and amylopectin measured in mature seed and at different time points). (Waxy (IR65), low (IR24), intermediate (IR64), high (IR36) and very high (IR36ae) amylose classes)	IR65 : 0.42% IR24 : 0.31% IR64 : 1.08% IR36 : 1.06% IR36AE: 7.96%	High High Intermediate Intermediate Low	0.46% 4.93% 8.04% 12.24% 11.77% *Inversely correlated with GI	Long chain amylopectin chain behaving like amylose 4.51% 5.62% 7.00% 8.74% 13.95% *Inversely correlated with GI
Kumar et al. (13)	Rice alone (*Oryza sativa L.*)	Max.: 2.93 % (Gayatri) Min.: 0.28 % (Hue)	Max.: 76.4 (Hue) Min.: 57.5 (Shaktiman)	Max.: 27.36% (Lalat MAS) Min.: 13.42% (Savitri)	N/A
	Rice-food combinations	Maximum effect and significant increase seen in pigeon pea, ghee oil, soybean oil, fenugreek, cauliflower-rice combinations	Maximum effect and significant decrease seen in pigeon pea, ghee oil, soybean oil, fenugreek, cauliflower-rice combinations)	N/A	N/A
Pongjanta et al. (14)	12 rice flour varieties, comprising each of five bran colors	White: 3.06 – 3.97% Brown: 3.27 – 6.13% Red: 3.20 – 5.50% Purple: 5.33 - 5.69%.	White: 78.92 – 83.59 Brown: 73.72 – 82.79 Red: 69.64 – 80.40 Purple: 68.50 – 75.20	White: 5.62 - 28.90% Brown: 5.73 - 19.30% Red: 5.40 - 21.92% Purple: 1.20 - 14.45%	N/A

Raigond et al. (15)	Cooked tubers of 46 Indian potato cultivars	Ranged from 1.22 to 1.93 mg/100 mg DW. Min: Kufri Bahar (1.22 mg/100 mg DW) Max: Kufri Garima (1.93 mg/100 mg DW)	N/A	Ranged from 10.8 to 27.6 mg/100 mg DW. Min: Kufri Giriraj (10.8 mg/100 mg DW) Max: Kufri Chipsona-3 (27.6 mg/100 mg DW)	N/A
Daley et al. (18)	Breadfruit cultivars	Overall mean: 46.03 ± 0.56 g/100g Highest: 50.53 ± 0.30 g/100g (Ma'afala' cultivar)	N/A	N/A	N/A
Naidoo et al. (19)	Amadumbe flour and starch	Wild amadumbe RS: 64%. Cultivated type RS: 57%).	Cultivated Raw: 42.1 Boil: 43.3 Bake: 44.0 Wild Raw: 41.0 Boil: 40.4 Bake: 43.0	Cultivated amadumbe flour: 12.0 Wild amadumbe flour: 20.0	N/A
Shumoy et al. (20)	Starch digestibility of injera and porridge	Bosset 32.2 Dega 16.9 Quncho 33.7 Simada 62.9 Tsedey 68.4 Zagurey 65.1 Zezew 57.6	N/A	Bosset 28.7 Dega 30.3 Quncho 30.6 Simada 29.0 Tsedey 30.6 Zagurey 30.0 Zezew 30.0	N/A
Guo et al. (21)	Investigated the loss-of-function mutants of SBEIIb using low-glutelin rice cultivars as recipients.	RS content reached approximately 6%	N/A	Increased by approximately 1.8-fold	N/A
Itoh et al. (22)	Novel mutant rice lines: to observe changes in RS content	The new mutant lines' RS content was similar to or slightly higher than the be2b parent line.	N/A	The introduction of granule-bound starch synthase (GBSSI) from the parent line significantly increased amylose content in the endosperm starch by approximately 10%.	

Noro et al. (23)	New amylopectin long-chain rice cultivar Chou 2418 and its boiled rice grains	Boiled Chou 2418: 5.30% - significantly higher than other cultivars. Boiled and stored Chou 2418 at 5°C, 24h: 13.9%	N/A	Apparent amylose content (AAC): 56.8% (significantly higher)	N/A
Slade et al. (24)	To identify novel genetic variation using TILLING in each of the A and B genomes in tetraploid durum wheat and the A, B and D genomes in hexaploid bread wheat, mutations in the form of single nucleotide polymorphisms (SNPs) in starch branching enzyme IIa genes (SBEIIa).	RS (%) PAA Lot1 vs Lot2 Durum WT: 1.58 vs 0.81 High Amylose: 6.21 vs 4.71 Bread WT: 0.83 vs 0.48 High Amylose: 11.21 vs 5.35 Purified Starch: 6.46 vs 6.32	N/A	Durum Parent:nd SBEIIa WT: 24.4 SBEIIa mutant: 47.4 Bread Parent: ND SBEIIa WT: 22.9 SBEIIa mutant: 55.7	N/A
Raja et al. (25)	EcoTILLING by sequencing of candidate genes	Variants in 3 SS genes: 7.5 to 7.6% Variants in 2 SS genes: 6.8 to 7.4% Variants in 1 SS genes: 4.1 to 6.1%	N/A	Os-076 26.0 Os-468 22.8 Os-578 24.5 Os-631 25.8 Os-678 27.2 Os-351 23.5 Os-211 23.5 Os-363 24.0 Os-495 23.7 RSM 271 23.3 RSM 311 24.2 Pooja 23.2	N/A

Yang et al. (27)	Verify and elucidate the mutated gene (sbe3-rs) for resistant starch in rice 'Jiangtangdao1'	Parent 'Jiangtangdao1': 11.67% Parent Miyang 23: 0.41% F3 generation families: 0.4 – 13.67% (majority were in the low RS region, 0.4-1.0%) Mutated high RS locus was recessive to regular-low RS.	N/A	Parent 'Jiangtangdao1': 31.1% Parent Miyang 23: 15.13%	N/A
Song et al. (28)	Low sugar cookies formulation using butter, xylitol, and high-amylose maize flour (HAMF) with optimal amounts of three ingredients are 183 g, 150 g, and 341 g respectively.	Butter, xylitol, and HAMF all positively related to the RS content of cookies	N/A	Higher proportions of long amylose chains shown on GPC weight distributions, W(logVh) curve.	Higher proportions of long amylopectin chains and lower short amylopectin chains shown on GPC weight distributions, W(logVh) curve
Hussain et al. (29)	Muffins formulated from water chestnut flour (WCF) and barley flour (BF)	WCF: 40.24% BF: 5.18% Muffins (70WCF:30BF): 43.5%	30.21 45.21 30.11	N/A	N/A
Hussain et al. (30)	Formulation of cracker: water chestnut flour (WCF) plus barley flour (BF)	WCF: 40.24% BF: 5.18% Crackers (70WCF:30BF): 44.5%	30.21 45.21 30.16	N/A	N/A
Lestari et al. (31)	My Cookies®: -arrowroot cookies supplemented with cinnamon and porang glucomannan	2.23 g/100 g	48.2	N/A	N/A

Zheng et al. (39)	Rice cultivar "Hefengyouzhan" (*Oryza sativa L.*) treated with optimized autoclave preparations (41.63% water content, pH 5.95, autoclave time 60.96 min, refrigeration time 17.11 hr)	Max. of 17.57%	Reduced from 78.35 to 66.08 (cooked rice)	N/A	N/A
Guha et al. (40)	Rice subjected to hydrothermal processing: − to observe changes in its physico-chemical and functional characteristics, the RS content and in-vitro digestibilty	Native rice: 0.23% Processed:0.60%	N/A	Native rice: Total amylose: 24.9% Soluble amylose: 12.7% In-soluble amylose: 12.2% Processed rice: Total amylose: 23.7% Soluble amylose: 9.2% In-soluble amylose: 14.5%	N/A
Zphoun et al. (41)	Rice varieties subjected to various parboiling steaming time at atmospheric pressure.	RS increased with increasing steaming time for NERICA1, IR841 and WITA4 NERICA7: - nonparboiled RS of 10.07% (highest among all) dropped to 2.49% after 25 minutes of steaming.	N/A	N/A	N/A
Saidu et al. (42)	Raw and boiled mushrooms: − Russula virescens − Auricularia auricula-judae − Sweet potato (*Ipomea batatas*) − Potato (*Solanum tuberosum*)	Raw RV: 0 Boiled RV: 2.20% Raw AAJ: 0 Boiled AAJ: 3.5% Raw unpeeled ST: 10.10% Boiled unpeeled ST: 4.50% Raw peeled ST: 5.60% Boiled peeled ST: 7.10% Raw IB: 2.20% Boiled IB: 0	N/A	N/A	N/A

Widjajaseputera et al. (45)	Mung bean subjected to different soaking time	(g/100 g) 0 hr : 11.12 ± 0.10 2 hrs: 8.33 ± 0.09 4 hrs: 18.49 ± 0.27 6 hrs: 14.49 ± 0.06 8 hrs: 13.65 ± 0.19	N/A	(g/100 g) 32.56 ± 0.31 33.09 ± 1.18 33.06 ± 3.42 34.55 ± 0.43 35.22 ± 3.25	N/A
Ye et al. (47)	Extruded rice (at 3 different level of moisture) starch treated with β-amylase	RS increased (48.74% to 77.62%) for extruded and enzymatically treated sample with increasing moisture content. RS decreased (19.89% to 6.92%) for extruded sample with increasing moisture content.		N/A	N/A
Tangjaidee et al. (48)	Selenium, fibre, and protein enriched rice product subjected to extrusion	Enriched extruded rice: 12%	Enriched extruded rice: 68.33 Extruded rice: 81.98	N/A	N/A
Kannayiram et al. (49)	Senna Auriculata Fortified-Bread (SAFB)	SAFB 2%: 4.76 ± 1.38% 4%: 5.04 ± 1.76% 6%: 10.55 ± 1.20%	58.11 45.18 50.13	N/A	N/A
Reshmi et al. (50)	Breads incorporated with pomelo fruit	Pomelo incorporated bread RS fractions: (3.87-10.96%)	Pomelo incorporated bread predicted GI: (62.97-53.13%)		
Giri et al. (51)	Indian traditional foods formulations with addition of guar gum, oats, barley and gluten	Increased in the content of all the four ingredients increased the resistant starch	− With an increase in the quantities of additional components, a linear drop in GI was observed. − Guar gum and oats were more effective.	N/A	N/A

Ahmed et al. (52)	Formulation of low-cost wheat based chapati flours with added functional ingredients: oat, barley and psyllium Control: wheat flour (100%)	Control: 26.91±2.30 WPOB-I: 22.69±1.97 WPOB-II: 18.95±1.77 ** − WPOB-I: − Wheat flour (75%); psyllium (5%); oat (10%); barley (10%) − WPOB-II: − Wheat flour (60%); psyllium (10%); oat (15%); barley (15%)	N/A	N/A	N/A
Quek et al. (53)	The in vitro digestibility of white rice (WR) cooked with lingonberry (WRLB), cranberry (WRCB), and red grape (WRRG)	− There was a positive correlation between the phenolic content of red grapes and RS (R = 0.9854). − The addition of higher amounts of red grape polyphenols to white rice resulted in increased RS generation.	N/A	N/A	N/A
Menon et al. (54)	Formulation of low GI sweet potato spaghetti with addition of gums and fibre.	Gum fortified spaghetti (g/100g starch) Control: 30.90 Min: LBG1 (0.5%):43.49 Max: XG3 (1.5%): 53.52 Fiber + gum fortified spaghetti (g/100 g starch) Control: 30.9 Min: AF1 (10%):41.11 Max: WF2 (20%):48.65	The estimated GI for control spaghetti was 66.62, which could be decreased to 58–60 and 57–59 through 1.5% gum 20% fiber + xanthan gum fortification	N/A	N/A

Zhang et al. (57)	Grape seed proanthocyanidins (GSP)-potato starch complexes	Increased significantly from 12.32% to 21.67% with increasing binding of GSP	N/A	N/A	N/A

*N/A: not available

Table 2. The antidiabetic properties (inhibitory action against α-amylase and α-glucosidase) of different sources of food.

Reference	Formulation/ source/ treatment	Antidiabetic properties	
		α-amylase	**α-glucosidase**
Kannayiram et al. (49)	*Senna Auriculata* Fortified-Bread (SAFB)	77.67% inhibition at 100 μg/ml of *S. auriculata* extract	> 50% inhibition at 100 and 500 μg/ml of *S. auriculata* extract
Raigond et al. (15)	Cooked tubers of 46 Indian potato cultivars	Found only in cultivar Kufri Frysona (20.5%) amongst all the tested cultivars.	The percentage (%) of α-glucosidase inhibitory activity in 14 potato cultivars: Kufri Anand:17.6 Kufri Arun:1.7 Kufri Khasigaro:46.0 Kufri Kuber:52.8 Kufri Kundan:0.6 Kufri Muthu:40.5 Kufri Naveen:35.7 Kufri Neela:26.7 Kufri Pushkar:31.6 Kufri Red:7.8 Kufri Sadabahar:8.8 Kufri Safed:8.5 Kufri Satlej:0.3 Kufri Swarna:16.3
Saidu et al. (42)	Raw and boiled mushrooms: - *Russula virescens* - *Auricularia auricula-judae* - Sweet potato (*Ipomea batatas*) - Potato (*Solanum tuberosum*)	Inhibition activity: Raw RV : −121.22% Boiled RV : 0 Raw AAJ : 19.75% Boiled AAJ : −1.95% Raw unpeeled ST : 39.02% Boiled unpeeled ST : 62.20% Raw peeled ST : 18.54% Boiled peeled ST : 50.24% Raw IB : 64.14% Boiled IB : −131.39%	N/A
Patel et al. (36)	Experimented with enzyme kinetics to observe how starch retrogradation influences amylolysis catalysed by porcine pancreatic amylase.	Retrograded starch decreased catalytic efficiency of porcine pancreatic amylase (PPA)	N/A

Starch modification

Starch retrogradation is a chemical reaction in which disrupted amylose and amylopectin chains realign into a more ordered structure upon cooling and become resistant to α-amylase digestion, forming RS. The extent of starch retrogradation is largely determined by temperature and storage time. Retrogradation is usually rapid at first and later begins to slow down. Apart from that, the rate and extent of starch retrogradation are also influenced by the amount of water present during the retrogradation process (36,37). Physical modification of starch is a method that uses this concept to alter starch digestibility, requiring the presence of two elements: heat and water to effect changes in the starch molecular structure. Among the commonly used methods are heat, extrusion, microwave radiation, high pressure and hydrothermal treatment like heat moisture treatment (HMT) (38). A lot of starch modification research has been done to investigate changes in RS content by using this principle which was carried out by different methods, either physically, chemically or enzymatically. In one study by Zheng and his team (2020), RS content in rice "Hefengyouzhan" (*Oryza sativa L. cultivar*) has been demonstrated to improve through the autoclave method. In this study, the RS content was found to increase with increased autoclave treatment time. However, it started to decrease when the autoclave treatment time exceeded 60 minutes. The optimum treatment condition was obtained at an autoclave time of 61 minutes with a water content of 41.63%, a pH of 5.95, and a refrigeration time of 17.1 hours with a maximum RS content of 17.57%. Autoclaving the rice has also resulted in a decreased GI of the cooked rice (66.08) when compared to its untreated counterpart (78.35) (39). The boiling of the previously mentioned ALC rice, Chou 2418, also found to have the highest RS content (5.30%) immediately after cooking when compared to other cultivars, and it continued to increase (13.9%) when stored at 5°C for 24 hours as a result of retrogradation process (23). Guha et al. (40) investigated the effect of parboiling (hydrothermal processing method) on the RS content of rice where they found the RS content of the processed rice was almost 3-fold higher than the native rice. Despite the fact that this processing method was able to raise the RS content of the rice, the total amylose content was found to be slightly lower than the native rice. However, its in-soluble amylose was slightly higher. This might be due to the gelatiniseded starch's amylose macromolecules reassembling during slow drying (40). In another similar study, Zohoun et al. (41) found that the RS content of two uplands (NERICA1 and NERICA7) and two sativa (IR841 and WITA4) rice varieties changes with different parboiling steaming times at atmospheric pressure. The four varieties of rice were parboiled using a grain quality enhancer, energy-efficient and durable material (GEM) parboiler following procedures by Ndindeng et al. (2015) and later subjected to different steaming times (5, 15, 25, 35, and 45 minutes).

In this particular study, they found that the RS content of parboiled NERICA1, WITA4, and IR841 rice increased with an increase in steaming time. However, the opposite was found for NERICA7 as the non-parboiled rice was found to have a higher RS content (10.07%) compared to its parboiled counterpart (2.49%) at 25 minutes of steaming time (41).

In another study on two varieties of mushroom (*Russula virescens* and *Auricularia auricula-judae*), sweet potato (*Ipomea batatas*) and potato (*Solanum tuberosum*), Saidu et al. (42) investigated the changes in RS content when subjected to boiling. In this research, they found boiling of the two varieties of mushroom resulted in a significant increase in the RS content. On the contrary, the opposite occurs for unpeeled potatoes and sweet potatoes, where boiling has resulted in a significant decrease in the RS content. However, when they compared the effects of boiling between peeled and unpeeled potatoes, boiled peeled potatoes showed higher RS content despite having lower RS before subjected to boiling.

Legumes or beans are nutritious seeds that serve as food sources containing a significant amount of fibre, carbohydrates, protein, B vitamins, iron, copper, magnesium, manganese, zinc, and phosphorous. Due to its low GI property, it is considered a healthy choice of food that produces lower postprandial glycaemic responses (43,44). Widjajaseputera et al. (45) investigated the effects of soaking in distilled water on the carbohydrate profiles of mung beans where they used time as a single factor tested at five different levels. They found that the RS content was significantly ($p<0.05$) higher than the unsoaked counterparts after 4 hours of soaking but started to decrease when soaked longer. However, the soaking procedure did not seem to have a significant effect on the amylose content of the mung bean.

Apart from the method used above, thermomechanical and enzymatic modification of starch has also been demonstrated to lower rice starch digestibility hence increasing its RS content. Extrusion of starch in the presence of sufficient water induces a number of physicochemical and functional changes in starch granules, including the loss of granular structure due to the melting of crystallites and underlying helices, as well as the formation of an amorphous structure (46). Ye et al. (47) pre-gelatinised rice starch through the extrusion method and further treated it with β-amylase. The effect of extrusion was studied at different moisture levels and they found that an increase in the moisture of feed during extrusion resulted in an increased degree of rice starch gelatinisation thus leading to better penetration of starch granules by β-amylase. The enzymatically treated extruded samples also showed an increase in RS content along with an increase in moisture content. The enriched product contains 12/100 g RS from yam and konjac flour substitution. In another study, Tangjaidee et al. (48) enriched rice products with a predetermined percentage of yam, konjac, sorghum and soy protein through the extrusion method. The enrichment has resulted in rice products with a much lower GI value (69.9 vs 95.4) when compared to commercial rice which contains 12% RS.

Food addition is another method being used to study starch modification in food or food products. Tangjaidee et al. (48) conducted a study on bread fortified with *Senna auriculata* (SA) to evaluate the starch digestibility as well as the GI of the bread product. It was reported that the RS content of the bread product increased with an increased percentage of SA extract being fortified in the bread with the value ranging from 4.76 to 10.55% and the lowest GI value of 45.18 was achieved with fortification of 4% SA extract (49). Apart from that, incorporating bread with pomelo fruit (*Citrus maxima*) segments had also been reported to increase levels of RS fractions (3.87–10.96%) with a low predicted glycaemic index (62.97–53.13%) in the bread product (50). The addition of pulses, cooking oil and vegetables has also been reported to have a significant effect on the GI and RS content of Asian rice (*Oryza sativa L.*). In this study, a dialysis membrane was used as a simulation of enzymatic digestion in the small intestine and they found pigeon pea, ghee, fenugreek and the cauliflower-rice combination resulted in a significant decrease in GI and increase in RS content of the rice (13). Another study by Giri et al. (51) investigated the effect of the addition of barley, oats, gluten and guar gum on starch digestibility and GI of Indian traditional food: idli, dosa, upma and chapatti formulations. Starch digestibility study of the four food products with and without added ingredients showed an increasing pattern of RS content as the concentration of the added ingredients increased. As for GI, increasing the concentration of added ingredients resulted in a decrease in GI values of the food products. Oats and guar gum were found to be more effective in reducing starch digestibility and GI when compared with two other ingredients. Ahmed et al. (52) in their study formulated low-cost wheat-based composite chapati flours containing barley, oat and psyllium as functional ingredients since chapati is widely consumed in most parts of India. Composite flours were formulated using psyllium, barley and oat at two different levels [WPOB-I = wheat flour (75 %), psyllium (5 %), oat (10 %) and barley (10 %), WPOB-II = wheat flour (60 %), psyllium (10 %), oat (15 %) and barley (15 %)]. However, the two formulations resulted in reduced RS content when compared to control despite having better profiles for other starch digestibility characteristics (52).

Following the evidence that plant polyphenols exhibit similar effects to insulin in reducing blood glucose and inhibiting α-amylase and α-glucosidase in vitro, Quek et al. (53) explored the effect of boiling white rice with lingonberry, cranberry and red grape to observe the ability of their polyphenols to retard enzyme activity thus lowering the rate of digestion. The results showed that combining red grape polyphenol with white rice showed the greatest impact on in vitro digestibility at 7% concentration. Red grape polyphenol was also reported to increase RS content in a dose-dependent manner. A similar impact was seen in spaghetti when cooked in addition to edible gums and fibres in a study that evaluated the comparative effects of edible gums: guar, xanthan, and locus bean gums, and dietary fibres: apple, oat, and wheat fibres in reducing starch digestibility and GI. Gums with low levels of incorporation (0.5–1.5%) generated spaghetti with medium GI, and xanthan gum outperformed the other gums as well as its combination with fibre sources. The 1.5% gum fortified spaghetti had the highest RS, and among the gums, xanthan gum produced cooked spaghetti with the highest RS content (53.52%) (54). Zhang et al. (57) in another study modified the starch digestibility of potatoes by complexing it with proanthocyanidins from grape seed (GSP), which has previously been shown to have potential anti-diabetic properties (55). They found that increased binding of GSP resulted in a significant increase in RS content (from 12.32% to 21.67%). Apart from starch modifying properties, GSP also had an inhibitory effect on digestive enzymes (56,57).

Antidiabetic properties

Apart from its low GI property which is beneficial in terms of reducing blood glucose spikes and insulinaemia, RS present in certain foods also exhibits inhibitory activity on digestive enzymes. The factors that enable RS to resist attack by digestive enzymes are the physical and chemical characteristics of the RS itself. In RS1, the compact molecular structure of the starch and the trapping within undamaged cell walls limit accessibility by digestive enzymes. The resistance of RS2 to the activity of the digestive enzyme is primarily correlated to the structure of the native starch granules, especially the B- and C-type polymorhs. Retrograded starch or RS3 is able to escape digestion by digestive enzymes due to the formation of starch crystals that are resistant to digestion during the retrogradation process. During this process, gelatinised starch is stored and allowed to cool causing it to acquire a more ordered structure that resists enzyme attack. RS4, on the other hand, resists enzyme digestion because it has been modified chemically by etherisation, esterisation or cross-bonding process. The molecular structures of the complexing lipid and the amylose in RS5, as well as the crystalline structure of the amylose-lipid complex, influence enzyme resistance in the amylose-lipid complex. The amylose-lipid combination has also been reported to prevent granule swelling in starch granules when heated in excess water, hence, reducing enzyme accessibility to hydrolyse the starch granules (6,58,59,60).

α-Amylase and α-glucosidase are enzymes found in the digestive tract responsible for breaking down the α-1,4 and α-1,6 glycosidic linkages in amylose and amylopectin of starch to yield glucose monomers which will then be absorbed into the bloodstream and, hence, lead to a postprandial increase in blood glucose. Starch that can bypass digestion by these two enzymes is considered a good starch alternative for individuals with impaired glucose metabolism as it lowers the rate of digestion and absorption into circulation. As a result, the postprandial blood glucose is maintained at a lower level which in turn leads to decreased insulin demand (61,62). Hence, there has been a lot of interest in searching or modifying food or food products that exhibit these properties. The SA extract used by Kannayiram et al. (49) in research on the previously mentioned fortified bread did exhibit these characteristics. The extract showed 78% of α-amylase inhibition at 100µg/ml, while more than 50% α-glucosidase inhibition activity was seen at 100 and 500 µg/ml of the extract. Screening of these two properties in 46 Indian potatoes revealed that only one cultivar exhibited α-amylase inhibition activity while 14 cultivars showed varying degrees of α-glucosidase inhibition activity ranging from 0.3 to 52.8% (15). In a different study, boiling of food samples has been shown to either increase their α-amylase inhibition activity or stimulate more α-amylase digestion in food samples when compared to their raw counterparts (42). Patel et al. (36) embarked on a study to evaluate the effect of retrogradation on amylolysis by porcine pancreatic amylase (PPA). They discovered that the degree of catalysis by PPA decreased with increasing evidence of starch retrogradation; hence, they deduced that RS directly inhibits PPA activity. The low GI and low digestibility rate of RS can benefit those with impaired glucose metabolism as it allows sustained and reduced glucose monomers to be released into circulation. This will not only help to improve post-prandial glycaemic response but also insulinemic response.

CONCLUSION

In conclusion, RS levels in food are greatly influenced by the source of the starch itself, the processing method used, conditions during processing, chemical alteration of the starch and the starch storage conditions. Apart from that, the genetic modification has also been proven to increase RS content. This may serve as a theoretical foundation for the future development of safe, healthy and nutritious cultivars of staple foods that can benefit people. Despite all the good traits evident in the food analysed in these studies, any generalisations for a particular modification method, food, or RS category could not be drawn because the studies in this review were conducted utilising a variety of methods and foods. Further in vivo and clinical studies are also warranted in order to evaluate and understand the RS mechanism involved in the effective management of blood glucose levels in diabetes.

CONFLICTS OF INTEREST

All authors have read and agreed to the published version of the manuscript. The authors declare no conflict of interest.

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