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COMPREHENSIVE REVIEW

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Resistant starch—An accessible fiber ingredient acceptable to the Western palate

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Abstract

Dietary fiber intakes in Western societies are concerningly low and do not reflect global recommended dietary fiber intakes for chronic disease prevention. Resistant starch (RS) is a fermentable dietary fiber that has attracted research interest. As an isolated ingredient, its fine particle size, relatively bland flavor, and white appearance may offer an appealing fiber source to the Western palate, accustomed to highly refined, processed grains. This review aims to provide a comprehensive insight into the current knowledge (classification, production methods, and characterization methods), health benefits, applications, and acceptability of RS. It further discusses the present market for commercially available RS ingredients and products containing ingredients high in RS.

The literature currently highlights beneficial effects for dietary RS supplementation with respect to glucose metabolism, satiety, blood lipid profiles, and colonic health. An exploration of the market for commercial RS ingredients indicates a diverse range of products (from isolated RS2, RS3, and RS4) with numerous potential applications as partial or whole substitutes for traditional flour sources. They may increase the nutritional profile of a food product (e.g., by increasing the fiber content and lowering energy values) without significantly compromising its sensory and functional properties. Incorporating RS ingredients into staple food products (such as bread, pasta, and sweet baked goods) may thus offer an array of nutritional benefits to the consumer and a highly accessible functional ingredient to be greater exploited by the food industry.

KEYWORDS
resistant starch, dietary fiber, fermentable fiber, glycemic response, gut microbiome

1 | INTRODUCTION

Increased consumption of processed food has led to a reduction in average dietary fiber intakes and an associated chronic disease prevalence. Although dietary fiber recommended daily intakes for adults range between 21 and 38 g/day (see Table 1), average daily fiber intakes in Westernized societies are concerningly below these levels.

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The National Adult Nutrition Survey revealed that 81% of Irish adults do not consume the EFSA-recommended 25 g/day dietary fiber (IUNA, 2011). These findings closely mirror data from the UK National Diet and Nutrition Survey (2014–2016) in which mean adult intakes were 19 g/day, with only 9% of adults meeting the recommended intake of 30 g/day dietary fiber set by the United Kingdom's Scientific Advisory Committee on Nutrition (SACN) (Roberts et al., 2018). In agreement, the US National Health and Nutrition Survey (2009–2010) estimated that the mean dietary fiber intake for Americans is 16.2 g/day, with 95% of US adults and children not consuming the IoM-recommended amounts of fiber (Quagliani & Felt-Gunderson, 2017).

Dietary fiber has thus been identified as a nutrient of public health concern in the 2010 Dietary Guidelines for Americans (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020), and campaigns such as the UK Food and Drink Federation's "Action on Fiber" initiative aim to encourage product reformulation within the food industry to increase population dietary fiber intakes (Food & Drink Federation, 2022).

Resistant starch (RS) is a unique dietary fiber that has attracted research interest due to its distinct sensory (fine particle size, bland flavor, and white appearance) and physiological (e.g., fermentability, low viscosity) properties compared to traditional fiber sources. RS may be an attractive fiber enrichment tool that may offer a solution to help bridge the "fiber gap," (i.e., the gap between current fiber intakes and scientifically recommended intakes) which is ubiquitous in Western diets. Hence, there is a significant opportunity for food innovation and the development of palatable, fiber-enriched foods, such as those that incorporate RS to enhance dietary fiber intakes in those following a Western-style diet and achieve physiological health benefits.

1.1 | What is RS?

Starches are one of the primary forms of dietary carbohydrates, and foods high in starch (e.g., cereal products and potatoes) are a staple component of most diets. Chemically, starch is a glucose heteropolysaccharide composed of amylose and amylopectin (Bendiks et al., 2020). Amylose is present as α-1, 4-linked linear molecular chains with minor branching, and amylopectin is a branched polymer composed of approximately 95% α-1, 4, and 5% α-1, 6 linkages.

Concerning digestibility, starch may be divided into three classifications: rapidly digesting starch (RDS), slowly digesting starch (SDS), and resistant starch (RS) (Dupuis et al., 2014). RDS and SDS starch fractions are hydrolyzed to dextrins by α-amylase within 20–120 min after ingestion, respectively (Dupuis et al., 2014). In contrast, RS, which was first described by Englyst et al. (1982), is a starch fraction resistant to enzymatic hydrolysis by α-amylase and pullulanase in vitro treatments. It may be defined as the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals (Sanz et al., 2009). RS is not hydrolyzed to D-glucose in the small intestine within 120 min of consumption but is, for the most part, fermented in the colon (Fuentes-Zaragoza et al., 2011). RS thus meets the Codex Alimentarius Commission definition for dietary fiber, which includes carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans (Codex Alimentarius, 2021).

TABLE 1 Summary of dietary fiber recommended daily intakes from select institutions

Institution	Dietary recommendation	Age- and gender-specific dietary fiber recommended intake levels (g/day)		
		Age (years)	Male	Female
IoM (2005)	Adequate intake (AI)	9–13	31	26
		14–18	38	26
		19–30	38	25
		31–50	38	25
		>51	30	21
EFSA (2010)	AI	>18	25	25
SACN (2015)	Dietary reference values	2–5	15	15
		5–11	20	20
		11–16	25	25
		16–18	25	25
		>18	30	30

The RS that reaches the large intestine acts as a substrate for microbial fermentation. It produces gasses (hydrogen, carbon dioxide, and methane), lactate, succinate, bacterial cell biomass, and various short-chain fatty acids (SCFAs) (including acetate, propionate, and butyrate), which may positively influence intestinal health (Jiang et al., 2020). The colonic fermentation can also lead to a reduction in secondary bile acids, phenol, and ammonia, which may confer additional health benefits (Martínez et al., 2010). Further to its classification as a mostly fermentable fiber, other physicochemical properties associated with RSs include their typically low viscosity and solubility (see Table 2).

The health benefits RS may offer as a fiber source have been continuously explored since its discovery, and its applications to the functional food sector are being exploited by both the scientific community and the food industry.

2 | CLASSIFICATION OF RS

RS may refer to a diverse range of materials, and there are five generally accepted types of RS, RS1–RS5. Its definitions and subtypes are continuously expanding to reflect the latest scientific advancements. The nature of RS in foods may depend on the starch botanical source, the extent of processing, and storage conditions (Aigster et al., 2011). See Table 2 for a descriptive summary of the five types of RS and their susceptibility to digestion in the large intestine.

2.1 | RS type 1

Type 1 RS refers to starch physically inaccessible in starchy foods, which are not fractionated and refined (Sanz et al., 2009). This RS form may be entrapped within whole or partly milled grains or seeds (Fuentes-Zaragoza et al., 2011), for example, sorghum grain in addition to legumes, raw fruits, and vegetables (Gill et al., 2021). Human amylolytic enzymes do not have access to starch accumulated in intact plant cells as the gastrointestinal tract lacks enzymes capable of degrading the components of plant cell walls (Leszczyński, 2004). Therefore, this form of RS passes through the small intestine in its intact form.

Disruption of the food structure, for example, milling (or mechanical chewing), may enhance digestion by giving digestive enzymes access to the food matrix (Topping et al., 2003). RS1 is heat stable under most normal cooking conditions enabling it to be used as a component in various conventional foods (Fuentes-Zaragoza et al., 2011). As RS1 is difficult to isolate and purify, there is currently an absence of isolated RS1 ingredients on the

TABLE 2 Summary of the five types of resistant starch (RS), their physicochemical characteristics, and digestion in the colon. Adapted from Raigond et al. (2015)

RS type	Description	Food sources	Solubility	Viscosity	Fermentability	Digestion in small intestine
RS1	Physically inaccessible starch [1]	Whole or partly milled grains or seeds [2], legumes, raw fruits, vegetables [3]	Insoluble	Nonviscous	High	Slow, partial digestion, may be digested fully if properly milled
RS2	Native starch granules [1]	Green banana, raw potato, high amylose corn [1], cereals, raw legumes, raw fruits, vegetables [3]	Low	Nonviscous	High	Very slow rate of digestion in raw state. If fully cooked complete digestion may occur
RS3	Retrograded starch	Cooked and cooled potato, bread, cornflakes, foods exposed to repeated moist heat treatments [2]	Low	Nonviscous to low	High	Slow rate of partial digestion. Digestibility may improve by reheating
RS4	Chemically and/or physically modified to increase resistance [2]	High-fiber drinks, bread, and cakes with modified starches (e.g., acylated starches)	Low to high	Low to medium	Variable (high to low)	This form of RS may resist hydrolysis
RS5	Amylose–lipid complexes	Foods with high amylose. Starch and lipid containing cereals. Synthesised, for example, steric acid -complexed high amylose starch [4]	Low	Low	Low	Can resist digestion

References: [1] Sanz et al. (2009), [2] Fuentes-Zaragoza et al. (2011), [3] Gill et al. (2021), [4] McCleary et al. (2021)

market. Thus, the potential benefits of RS1 are challenging to research in isolation as they are typically ingested in combination with other fibers and phytonutrients.

2.2 | RS type 2

Native RS granules, which contain uncooked starch or starch that was poorly gelatinized, may be classified as RS type 2 (Fuentes-Zaragoza et al., 2011). This type of RS is mostly composed of linear amylose chains (with minor branching) and may be present in green bananas, raw potatoes, and high-amylose maize starch (Sanz et al., 2009). The crystallinity of these starch structures make them resistant to enzymatic hydrolysis. Moreover, these ungelatinized starches are resistant to digestion because of their compact structure (Ashwar et al., 2016).

Starch granules from tubers are among the most hydrolysis-resistant native starches (Dobranowski & Stintzi, 2021). The resistance of raw potato starch may result from the large size of its granules, which limit the area availabile to digestive enzymes (Leszczyński, 2004). The specific surface area of potato granules is smaller than cereal starches. There is a lower degree of enzyme adsorption on the surface of potato granules, contributing to the lower degree of amylolytic degradation (Leszczyński, 2004). The digestibility of raw starches, for example, in unripe bananas, is increased through cooking and heat treatments, especially in water, which gelatinizes the starch and provides greater access to amylases (Topping et al., 2003).

2.3 | RS type 3

The third classification of RS refers to retrograded starch which may be processed from unmodified starch or result from food processing applications. The retrogradation process occurs when starch undergoes gelatinization, followed by a thermodynamically driven reconfiguration of amylose and amylopectin into a new ordered state (Dobranowski & Stintzi, 2021). Although cooking can initially increase unmodified starch digestibility, subsequent heat treatment can lead to the formation of crystallites resistant to digestion (Topping et al., 2003). The native starch structure may be retrograded spontaneously or artificially precipitated from the starch paste, which occurs in water-insoluble, semi-crystalline structures. Due to the retrogradation process, more thermostable structures are formed by amylose instead of amylopectin (Leszczyński, 2004).

Amylose is slowly digested in the gastrointestinal tract, whereas amylopectin is digested rapidly after retrogradation (Raigond et al., 2015). Higher amylose content is thus associated with higher resistance toward enzyme digestion, which may be attributed to the compact mostly linear structure of amylose and the presence of hydrogen bonding linking the glucose chain of the starch amylose (Zaman & Sarbini, 2016). It has been observed that the RS3 content of food appears to increase with repeated heating and cooling cycles (Topping et al., 2003). Food sources of RS3 include cooked and cooled potatoes, bread, and food products exposed to prolonged or repeated moist heat treatment (Fuentes-Zaragoza et al., 2011).

2.4 | RS type 4

Type 4 RS represents starch chemically or physically modified to obtain resistance to enzymatic digestion (e.g., some starch ethers, esters, and cross-linked starches) (Fuentes-Zaragoza et al., 2011). These modifications introduce bulky functional groups, for example, hydroxypropyl, acetyl, and octenyl succinic anhydride groups, or linkages between amylose chains, for example, through phosphate moieties (Roman & Martinez, 2019). The chemical modifications inhibit the digestion of starch by preventing access to enzymes and forming atypical links (Ashwar et al., 2016). As the functional groups are added by substitution along the α-1, 4 D-glucan chains, they inhibit enzymatic degradation, making adjacent glycosidic bonds inaccessible to the enzymes (Roman & Martinez, 2019).

The presence of cross-linked starch chains inhibits granular swelling, preserves structural integrity, and contributes to steric hindrance, which prevents amylase from properly binding to starch (Roman & Martinez, 2019). RS4 does not occur in nature. Still, it can be added to foods such as high fiber drinks, bread, and cakes. RS4 is typically used for formulations that require smoothness, pulpy texture, flowability, low pH storage, and high-temperature storage (Yuan et al., 2018).

2.5 | RS type 5

Starch–lipid complexes, including starch–fatty acids, and starch monoglycerides, have been classified as type 5 RS (Gutiérrez & Tovar, 2021). Additionally, other starch-based complexes such as starch–glycerol, starch–amino acids, starch–peptides, starch–proteins, starch–lipid–protein, and starch–polyphenols may contribute to the RS5 content of foods (Gutiérrez & Tovar, 2021) and may be present in cereals containing starch and lipids.

Amylose–lipid complexes are most often discussed when referring to RS5 and are commonly present in native and processed starch. The enzyme resistance of these complexes may be attributed to the crystalline structure

of the amylose helical complex, which protects the bulk of the amylose from enzymatic hydrolysis (Hasjim et al., 2013). Additionally, the amylose–lipid complex present in starch granules may increase enzymatic resistance due to their ability to restrict the granules swelling during the cooking process (Hasjim et al., 2013). However, the degree of enzymatic resistance is highly variable and dependent on the molecular structure of the lipid and the crystalline structure of the single helices (Hasjim et al., 2013). At present, commercial RS5 sources are not available on the market (Roman & Martinez, 2019).

3 | HOW ARE RS INGREDIENTS PRODUCED?

The production of RS ingredients will vary depending on the type of RS under investigation. Modifying starch is the most important method to improve RS content (Jiang et al., 2020). The manufacture of RS isolates usually involves partial acid hydrolysis and hydrothermal treatments, heating, retrogradation, extrusion cooking, chemical modification, and repolymerization (Fuentes-Zaragoza et al., 2011). The content of RS formed is dependent on the severity of processing conditions such as temperature, pH, moisture content, and the number of heating and cooling cycles implemented. When processed, the native types of RS (e.g., RS1 and RS2) are often destroyed. RS levels in food have been shown to increase during storage due to retrogradation and amylose chain crystallization (Aigster et al., 2011).

The isolation of RS using chemical methods may lead to limitations associated with low reaction rate, long production durations, unstable product quality, product safety, and environmental pollution (Jiang et al., 2020). In contrast, physical modification methods are more environmentally friendly, economical, and applicable, producing RS with superior physicochemical properties (Jiang et al., 2020). Physical modification methods include primary heat moisture treatment, autoclaving, and annealing and less invasive methods such as high hydrostatic pressure (HHP), microwave, extrusion, and sonification, which are more energy-efficient and less time-consuming (Jiang et al., 2020).

3.1 | RS1

There is currently a void of literature describing RS1 isolation techniques. To prevent a loss of RS1 during manufacture, one may aim to maintain the stability of the entrapping material and monitor the manufacturing conditions to maximize the trapped sources of enzyme-resistant starch within foods. Milling (e.g., of grains or seeds), for example, should be performed with care to prevent a loss of RS1 via damaging the tissue matrix (cell wall and protein network) (Roman & Martinez, 2019). To reduce the effects of milling, coarse milling may be preferred, or larger particles could be isolated after the fractionation process (Roman & Martinez, 2019).

3.2 | RS2

Raw green banana flour is a rich source of RS2 (52.7–54.2 g/100 g dry basis) (Tribess et al., 2009), and such flours may be produced by drying green banana pulp. The most common drying methods are sun drying and hot air drying (Ahmed, 2020). During manufacture, the drying conditions must be carefully monitored to ensure a rapid inactivation of hydrolyses involved in converting starch to reducing sugars during ripening (Pico et al., 2019). Additionally, the RS content may be better preserved when drying is performed at higher temperatures and air velocities to reduce drying time (Pico et al., 2019). Green banana flours may also be produced by other drying methods such as drying in a spouted bed, freeze-drying, and spray drying (Ahmed, 2020).

Other RS2s include high-amylose maize starch (HAMS), a popular commercial RS2 that may be formed due to endosperm mutations that modify the nature and ratio of amylose to amylopectin (Thompson, 2000). The majority of HAMS arise from the *amylose-extender* (*ae*) gene, and for *ae*-type maize starches, the level of amylose is highly variable depending on the genetic background (Thompson, 2000). The strategic choice of the genetic make-up of plants may be manipulated by manufacturers to alter RS2 levels within the plant (Thompson, 2000).

The isolation and production of RS2 rich ingredients may offer a more accessible way to expose consumers to the potential benefits of this fermentable fiber and may help overcome some of the sensory issues associated with native raw starches (e.g., unripe bananas and raw potatoes are considered less palatable [bitter flavor and hard, starchy texture] and more difficult to digest than their ripened or cooked counterparts).

3.3 | RS3

The production of type 3 RS may be achieved via the retrogradation of starch molecules and later gelatinization or dispersion of the native starch granules (Thompson, 2000). Retrogradation causes the polymer chains to reassociate via hydrogen bonds forming double helices (Ashwar et al.,

2016). RS3 is affected by the degree of polymerization (DP) of amylose; as the DP increases, the RS3 content increases to a maximum of 100 DP and after that remains constant. To form a double helix, the DP level must reach 10–100 (Ashwar et al., 2016). Selective hydrolysis may be performed before thermal treatments to increase polymer mobility for molecular rearrangement, and posttreatment hydrolysis may be performed to enhance the RS content of the ingredient (Thompson, 2000).

3.4 | RS4

Type 4 RSs may be produced via chemical modification methods, resulting in dextrinization, etherification, esterification, oxidation, and cross-linking (Roman & Martinez, 2019). Chemically induced cross-linking is a common technique used in the production of RS4s. Increasing the degree of cross-linking of starch granules may inhibit the entrance of α-amylase molecules into starch granules (Woo & Seib, 2002), thus aiding enzymatic starch resistance. Cross-linking intends to add intra- and intermolecular bonds at random locations in the starch granule that stabilize and strengthen the granule. Additionally, treatment of granules with multifunctional agents may contribute to the formation of ether or ester linkages between hydroxyl groups on starch molecules (Kahraman et al., 2015).

Woo and Seib (2002) prepared a series of cross-linked modified food starches via the phosphorylation of wheat, corn, high-amylose corn, oat, rice tapioca, mung bean, banana, and potato starches in aqueous starch slurries (approximately 33% starch solids w/w) with 1–19% (starch basis) of a 99.1 (w/w) mixture of sodium trimetaphosphate (STMP) (a cross-linking agent) and sodium tripolyphosphate (STPP) (a substituting agent) while maintaining an alkaline pH. The reactions were monitored for 0.5–24 h at 25–70°C with sodium sulfate or sodium chloride additions at 0%–20%. The starches formed are phosphorylated di-starch phosphodiesters. Fibersym® RW is a commercially available RS4 wheat starch produced by the method of Woo and Seib (2002) under US patent 5 855 946, and its chemical name is phosphate di-starch phosphate.

3.5 | RS5

Amylose–lipid complexes, which are naturally present in some starch sources (e.g., HAMS), may be formed via hydrothermal treatments, including baking, in the presence of lipid sources (including monoglycerides, fatty acids, lysophospholipids, and surfactants) (Roman & Martinez, 2019). Amylose–lipid complexes may, for example, be formed from high amylose starches that require higher temperatures for gelatinization and are more susceptible to retrogradation (Fuentes-Zaragoza et al., 2011). At present, commercial RS5 sources are not available on the market (Roman & Martinez, 2019).

4 | CHARACTERIZATION OF RS

Determining the RS content of food ingredients and processed foods is essential to provide accurate nutritional information to consumers and facilitate the application of substantiated health claims for the marketing of food products. The gold standard method of RS determination involves in vivo techniques, including the human ileostomy model (Englyst et al., 1996; Iacovou et al., 2017). This model analyzes the ileal digesta of adults with permanent ileostomies for starch content compared to the total amount of starch ingested during the study period (Roman & Martinez, 2019). However, these techniques are time-consuming, costly, require highly trained facilitators, and are ethically challenging (Iacovou et al., 2017). Moreover, there may be a high degree of interpersonal variation in starch digestion due to variability in chewing, hormone responses, enzyme activity, passage rate, individual health status, and so forth. (Roman & Martinez, 2019).

In vitro methods for RS determination aim to stimulate the gastrointestinal digestion of starch in foods. Methods developed by Englyst et al. (1982) have been widely discussed (and confirmed through studies with healthy ileostomy subjects; Englyst & Cummings, 1985) and involve RS and other non-starch polysaccharides to be first separated from enzyme hydrolysable starch (Perera et al., 2010) via α-amylase and pullulanase treatments. The RS may be solubilized in an alkali solution, separating it from other enzyme-resistant polysaccharides. Berry (1986) followed a similar procedure to measure RS. However, the initial heating step at 100°C (as described by Englyst et al. (1982)) was removed to replicate human physiological conditions more closely. Using this method (Berry, 1986), the measured RS contents of samples were much higher than the method described by Englyst et al. (1982) (McCleary & Monaghan, 2002).

A later study by Englyst et al. (1992) describes the characterization of RS by incubating samples with pancreatic α-amylase (PAA), amyloglucosidase (AMG), and invertase at 37°C for 2 h to measure the digestible starch fraction. The total starch content may be determined separately, and the RS content may then be calculated by subtracting the total starch content of a sample from the added sum of the calculated rapidly digestible starch and slowly digestible starch (Englyst et al., 1992). This method

has been validated by comparing the results with results obtained from the ileostomy model (Englyst et al., 1992). Although this method provides information to the analyst, it is laborious and has poor reproducibility if analysts are not extensively trained (McCleary & Monaghan, 2002).

Goni et al. (1996) later adapted Berry's procedure (1986) to develop a direct method to quantify RS in food. This method (which simulates the physiological conditions of the stomach and intestine e.g., pH and transit time) incorporates the removal of protein and digestible starch followed by the solubilization and enzymatic hydrolysis of RS which may ultimately be quantified as glucose released × 0.9. The addition of a protein removal step was incorporated to increase amylase accessibility to avoid starch–protein associations or the formation of glutenous lumps. This method (Goñi et al., 1996) may be advantageous as it is less time-consuming, provides more reproducible results and is less expensive and laborious than Englyst's method. The authors used in vivo data available in the literature for validation (Champ et al., 2003).

McCleary and Monaghan (2002) subsequently developed a robust and reliable RS measurement technique involving PAA and AMG treatment while maintaining a pH of 6 at 37°C for 16 h to imitate human digestion. This enzymatic treatment allows non-resistant starch to be solubilized and hydrolyzed to D-glucose. This is subsequently followed by an alcohol precipitation step used to terminate the incubation reaction. Results obtained via this method have been shown to closely correlate with those obtained in vivo with ileostomy patients (McCleary & Monaghan, 2002). This method has been successfully subjected to complete AOAC interlaboratory evaluation, including 37 laboratories (Megazyme, 2019), and became the AOAC method 2002.02 and AACC Method 32–40.01 for RS determination.

A rapid resistant starch (RAPRS) procedure and test kit have been developed by Megazyme in which the incubation step has been shortened to 4 h instead of 16 h to better reflect the time of residence of food in the small intestine (Megazyme, 2019). This popular method involves incubating samples in a shaking water bath linearly with saturating levels of purified PAA and AMG for 4 h. At 37°C. The reaction may then be terminated by adding an equal volume of ethanol or industrial methylated spirits (IMS, denatured ethanol); the RS may be recovered as a pellet on centrifugation.

RS pellets may then be washed twice by suspension in aqueous IMS or ethanol (50% v/v) and centrifuged. The resulting free liquid may be removed via decantation. The RS pellet may then be dissolved in 1.7 M NaOH and vigorously stirred in an ice-water bath while being magnetically stirred. The solution may be neutralized by adding an acetate buffer, and the starch may be quantitatively hydrolyzed to D-glucose with AMG. The D-glucose content can be measured with a glucose/peroxidase reagent (GOPOD) which thus provides a measure of the RS content of the sample. The non-RS fraction of the sample may also be determined by collecting the original supernatant and the washings, adjusting the volume to 100 ml, and measuring the D-glucose content with the GOPOD reagent.

RS is considered the most difficult dietary fiber source to quantify because the values obtained are dependent on the hydrolysis conditions used in the analytical technique employed (i.e., it is a kinetic measurement) (McCleary et al., 2021). Different methods result in different quantitative results due to variations in sample preparation, selected enzymes, and incubation periods (Lockyer & Nugent, 2017). Additionally, RS is generally measured in samples containing high levels of digestible starch, which can limit reproducibility. The development of reproducible methods to characterize and quantify RS types in foods and isolated fiber ingredients is essential. This knowledge will allow food composition tables and dietary analysis software to be updated to estimate the contribution of RS to daily fiber intakes and thus population exposure levels to RS.

5 | POTENTIAL HEALTH BENEFITS OF RS

Dietary fibers, including RS, have shown promising effects in nutritional intervention studies for reducing risk factors for metabolic diseases. RS has positively affected select biomarkers for metabolic disease, including glucose metabolism and blood lipid biomarkers (Wang et al., 2019; Yuan et al., 2018). Dietary supplementation with RS may also contribute to improvements in satiety and can positively affect the gut microbiome by inducing prebiotic effects and increasing microbial diversity.

5.1 | RS health claims

At present, there has been one approved health claim for RS by the EFSA (2011) and one approved Food and Drug Administration (FDA) health claim for Hi-Maize, a high amylose maize starch (RS2) isolated fiber ingredient produced by Ingredion in the United States. See Table 3 for a summary of approved health claims for RS.

5.2 | Glucose metabolism

The health effect most researched for RS is its potential to improve glucose homeostasis. It has been substantiated by

TABLE 3 Summary of approved health claims for resistant starch (RS)

Organization	RS type	Health claim	Premise
EFSA (2011)	All RSs	"Replacing digestible starch with RS induces a lower blood glucose rise after a meal"	High carbohydrate baked foods should contain at least 14% of total starch as RS, in replacement to digestible starch
FDA (2016)	Hi-Maize®260; RS2 ingredient derived from high amylose maize starch	"High-amylose resistant starch may reduce the risk of type 2 diabetes"	Can be used on packaging of conventional foods as defined by by the Code of Federal Regulations 101.14 (Health claims: general requirements) [1]

Reference: [1] Douglas (2016)

the EFSA (2011) with an approved health claim "replacing digestible starch with RS induces a lower blood glucose rise after a meal," in addition to an FDA-approved health claim for a reduced risk of type 2 diabetes.

Most of the studies supporting the EFSA health claim assessed postprandial glycemic and insulinemic responses after consuming common baked foods such as crackers, bread, and muffins. The EFSA Panel on Dietetic Products, Nutrition and Allergies concluded that based on the evidence presented, a cause and effect relationship has been established between the consumption of RS from all sources, when replacing digestible starch in baked foods, and a reduction of postprandial glycemic responses (EFSA, 2011).

The evidence underpinning these findings includes a recent systematic review and meta-analysis (Wang et al., 2019) of 13 clinical intervention trials (five randomized crossover trials and eight randomized controlled trials, $n = 428$), which examined the effect of RS supplementation (ranging from 10 to 45 g per day) on glucose, insulin, and insulin resistance in overweight or obese adults (with follow-up periods of 2–8 weeks). The study noted a reduction in fasting insulin concentrations (overall SMD −0.72; 95% confidence interval [CI] −1.13 to −0.31) in diabetic and nondiabetic trials and a similar reduction in both populations for fasting plasma glucose concentrations (overall SMD −0.26, 95% CI −0.5 to −0.02). In the overall analysis, RS supplementation reduced HOMA-B (SMD −1.2; 95% CI −1.64 to −0.77) and HbA1c levels (SMD −0.43; 95% CI −0.74 to −0.13). The meta-analysis by Wang et al. (2019) supports the findings from the EFSA and FDA health claims and highlights that RS supplementation can improve fasting glucose, fasting insulin resistance, and sensitivity in overweight and obese diabetic and nondiabetic individuals.

It is commonly accepted that the digestion of RS-containing food in the small intestine is much slower than food containing readily digestible starch (RDS). Therefore, the consumption of high RS food leads to a lower level of glucose release (Wong & Louie, 2017). This effect is noted by the glycemic index of a food, that is, the ranking system that organizes foods according to the change of glycemic response after ingestion. Decreases in starch digestibility of a food contribute to a slower rise in blood glucose after consumption, reflected by a lower glycemic index value attributed to a foodstuff.

Mechanistic studies in animal models support the blood glucose-lowering effects of RS. Zhou et al. (2015) noted significantly reduced blood glucose levels in streptozotocin-induced diabetic rats administered with RS2 (2 g daily, approximately 8% of total diet) compared to a control group. These effects may be attributed to an increased expression of genes involved in glucose metabolism (including glycogen synthesis genes *GS2* and *GYG1* and insulin-induced genes, *Insig-1* and *Insig-2*) which may promote glycogen synthesis and inhibit gluconeogenesis with an associated blood glucose lowering effect (Zhou et al., 2015).

These results may highlight a potential application of RS in improving meal to meal regulation of blood glucose and insulin levels. RS supplementation may thus offer a safe therapeutic therapy for maintaining normal glucose metabolism and a preventative therapy for T2DM and symptoms of metabolic syndrome. The development of RS-enriched products with the appropriate formulation can significantly reduce the glycemic index of a food compared to their conventional counterparts. Such product applications will be discussed later in the review, and these findings indicate the potential for high RS ingredients to be applied to specialty foods, for example, diabetic food products.

Although the evidence for a health benefit of RS in reducing postprandial glycemic responses has been sufficiently supported, at present, the number of human studies is quite limited. In addition, all the evidence for the EFSA and FDA health claims rely on data from

TABLE 4 Descriptive summary of commercially available resistant starch (RS) ingredients

Product name	Producer name	Product description (by manufacturer)	Product marketing claims	Applications	References
ActiStar™	Cargill	Resistant tapioca starch, RS4	Supplementary DF. Low calorie 0.25 kcal/g. Low water binding capacity	Wide variety of grain-based products. Breads, cakes, cookies, crackers, pizza, tortillas	(Cargill Incorporated, 2021)
Fibersym®RW	MGP® ingredients	Granular, RS4 resistant wheat starch	Low FODMAP certification. Recognized by FDA as a DF. Meets FDA guidelines for gluten free foods (<20 ppm gluten). 0.4 kcal/gram. Increase fiber content, lower caloric value. Can be produced gluten-free. Smooth texture, neutral and white color	Batters, breads, biscuits, muffins, pizza crust, frozen dough, puffed cereals, pasta, noodles instant drinks, and energy bars	(Ingredion, 2020)
Hi-Maize® 260	Ingredion	Derived from high amylose maize. 53% RS2 and 43% digestible starch	Improved energy balance and weight management support. Positive effects on digestive health and diabetes. Fewer kilocalories than flour (2.78 kcal/g), enhanced nutritional profile	Breads, pasta, noodles snacks, breakfast cereals, and breakfast drinks	(Ingredion, 2020)
Novelose® 3490	Ingredion	Tapioca-based RS4, minimum 85% DF. Also available in rice-based RS4 (Novelose® 8490) and potato-based RS3 (Novelose® 330)	Certified low FODMAP, excellent source of fiber, gluten free, non-GMO, not sourced from grain, reduced kilocalories in comparison to flour. Low impact on taste, texture, and color. Low viscosity and water-holding capacity, process tolerant	Baked goods, extruded and sheet snacks, breakfast cereals, nutrition and breakfast bars, pasta and noodles, tortillas, low FODMAP foods	(Ingredion, 2018)
Versafibe™ 1490	Ingredion	Food grade modified RS4 potato starch. Insoluble DF. Minimum 85% DF	Excellent source of fiber, gluten free, not sourced from grain, potential calorie reduction. Certified low FODMAP by Monash University. Recognized DF by the FDA. Little impact on taste, texture, color of products. Low water-holding capacity	Baked goods and snacks, breakfast cereals, pastas and frozen meals, biscuits, pretzels, and tortillas, low FODMAP foods	(Ingredion, 2015)

(Continues)

TABLE 4 (Continued)

Product name	Producer name	Product description (by manufacturer)	Product marketing claims	Applications	References
NuBana™ RS65 green banana flour	International Agricultural Group	Derived from green bananas. Minimum 65% RS2	Prebiotic fiber, non-GMO, nonhygroscopic, rich in potassium available in organic grade.	Supplement powders, bars, beverage powders, cold-fill beverages, raw vegan foods	(International Agricultural Group, 2018)
Green banana resistant starch	Natural Evolution™	Derived from green bananas source of RS2, combination of soluble, insoluble, and fermentable fiber	Gluten free, vegan, paleo, high fiber, paleo, 5HTP. No additives or preservatives. Prebiotic, multifiber. Rich in inulin, zinc, magnesium, phosphorous, manganese	May be added to smoothies, cereal, soup, water, juice, etc.	(Natural Evolution, 2019)
Organic green banana flour	Livekuna®	Produced from organic dried and milled green bananas. Source of RS2	Organic, non-GMO. Gluten and grain free. Suitable for ketogenic and paleo diets. Natural sweetener. Source of RS, prebiotic DF. High fiber, source of essential amino acids	All-purpose wheat flour alternative. Baking and cooking applications. May be added to smoothies	(Live Kuna, 2021)
Solnul™	MSP Starch Products Inc.	Unmodified potato starch >60% RS2	Supplement grade quality. FODMAP friendly, gluten free, glyphosate free, non-GMO, Kosher friendly. Supports GI health, IBS symptoms, healthy aging, metabolic health with prebiotic benefits. Neutral color, taste, and mouthfeel	Not given	(MSP Starch Products Inc., 2020)
MS Prebiotic®	MS Prebiotic	Contains Solnul™ an unmodified high RS2 potato starch. Insoluble in liquids. Loses effectiveness when heated over 60°C	Prebiotic and fiber supplement. Contributes to a healthy microbiome, increase energy, source of DF, suitable for low FODMAP diets. Only supplement grade prebiotic RS on the market. Increases butyrate production	May be stirred into beverages and immediately ingested. Can be added to smoothies, shakes, yoghurt. Not suitable for cooking, baking, hot beverages	(MSPrebiotic Inc., 2021)

Abbreivations: CV, cardiovascular; DF, dietary fiber; GI, gastrointestinal.

studies in which RS2 ingredients derived from HAMS were evaluated. Further studies with larger sample sizes investigating the effects of various types of RS from different plant origins should be analyzed to determine if these findings are applicable to other RS isolates.

5.3 | Satiety and appetite regulation

Appetite may be regarded as a subjective construct and thus cannot be measured directly. Indirect measurements include observing eating patterns/food intakes, questionnaires, and biological biomarkers (Mattes, 2007). Appetite may be subdivided into three components: hunger, satiation, and satiety (Mattes, 2007). Hunger may refer to the sensations that encourage food consumption and may be of metabolic, sensory, and cognitive origin. Satiation may be defined as the satisfaction of appetite during feeding that marks the end of eating (determining meal size and duration). Subsequently, satiety refers to the inhibition of hunger as a result of having eaten (Slavin & Green, 2007), which determines the intermeal period of fasting.

Dietary fibers may impact satiation by adding bulk and affect satiety due to their viscosity. It may be observed that diets low in energy and fat, which are typically recommended for obese people (Slavin & Green, 2007), are poorly satiating. The addition of fiber isolates such as RS to low calorie/low-fat foods may enhance satiety and act as a potential therapy for weight loss in overweight and obese individuals at risk of developing chronic disease.

A recent systematic review and meta-analysis of four randomized, placebo-controlled clinical trials ($n = 174$) (Amini et al., 2021) noted lower appetites in groups acutely supplemented with RS (RS 1, 2, or 3) compared to controls (by examining the area under the curve [AUC] of the participant's ratings of appetite [using the visual analog scale]; weighted mean difference [WMD] −1.375 mm min, 95% CI: −1.673 to −1.076). The meta-analysis included studies that matched the placebo with the meal/food intervention in terms of other macronutrients and reported the AUC in time points from 0 to 240 min after consumption. Overall, the influence of RS on appetite was noted to be greater when the acute dose was ≥25 g per test meal. This meta-analysis (Amini et al., 2021) illustrates that the dose of RS is an essential determinant for an effect on appetite suppression, though has some limitations, including the small sample sizes, self-reported appetite measurements, and the acute nature of the trials included.

It has been suggested that RS may suppress appetite by delaying gastric emptying, modulating gastrointestinal hormones, and delaying the suppression of postprandial blood glucose (Amini et al., 2021). Furthermore, RS consumption may contribute to appetite reduction by increasing the presence of SCFAs fermentation products. Zhou et al. (2008) noted a sustained day-long upregulation on glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) in rodents supplemented with RS. The study noted increased proglucagon and PYY gene expression following the fermentation and release of SCFAs in the lower gut (Zhou et al., 2008). Additionally, rats fed RS diets (30% w/w RS2) for 10 days had significantly lower body fat than control fed rats (diets had an equal energy density), though their food intake was similar (Zhou et al., 2008).

Human intervention trials examining the effects of RS supplementation over longer trial durations are also available in the literature. A double-blind, parallel, placebo-controlled trial of healthy adults ($n = 22$) (Hoffmann Sardá et al., 2016) investigated the effect of consuming ready-to-eat soup with individual servings of unripe banana flour (UBF) (8 g), rich in RS2 (5 g/8 g UBF) three times per week for 6 weeks versus a control (ready-to-eat soup and individual servings of a placebo, containing 2 g of maltodextrin). After thawing and heating, the individual servings of UBF or placebo (maltodextrin) were added to the frozen soups. The 15 g/wk UBF intake significantly reduced hunger and increased satiety parameters (indicated by a visual analog scale [VAS] and AUC of plasma ghrelin and plasma peptide tyrosine tyrosine (PYY) levels (performed before and after the intervention). Additionally, the supplemented versus control group noted a 14% reduction in energy (representing approximately 150 kcal) intake at two subsequent meals.

However, the effects of RS supplementation on appetite suppression over longer trial durations may be variable. A 12-week randomized controlled trial of prediabetic adults ($n = 59$) investigated the effects of supplementing 45 g/day of RS2 (Hi-Maize 260) versus an isocaloric placebo product (45 g/day rapidly digestible starch amylopectin, Amioca) (White et al., 2020). The RS2 and placebo supplements were consumed in either a prepared yogurt (approximately one third of supplements) or via packets mixed into their regular meals. Two sets of VASs were used to assess appetite ratings, the first at 0 and 12 weeks (at 15, 30, 60 , 90 , 120, and 180 min postingestion). Participants also completed a weekly VAS to assess average ratings of appetite during the previous week. It was observed that the weekly VAS produced very similar appetite ratings to the daily VASs. The trial did not note any effect on appetite or appetite-related gut hormones, GLP-1, PYY, and ghrelin after a standard mixed meal test (analyzed from blood samples collected 15, 30, 60, 90, and 120 min postingestion of the test meal). Additionally, no effect was noted on total energy, carbohydrate, protein, or fat consumption compared to placebo supplementation. Hence, this study reported no effect on RS2 supplementation on appetite

perception, appetite regulatory gut hormones, food, and macronutrient intake.

Overall, the effects of RS supplementation on satiety are quite variable. Although some positive results have been observed for RS supplementation in relation to increasing satiety indicators and reducing energy consumption, further human intervention trials are warranted to investigate the specific dosages and RS types required to significantly affect one's satiety parameters to establish a potential role for RS supplementation in weight management. RS ingredients may have a lower effect on satiety in comparison to soluble, viscous fibers (such as β-glucan, guar gum, and psyllium) which may enhance appetite regulation by promoting gastric distention and increasing satiation (Jovanovski et al., 2021).

5.4 | Blood lipid profiles

Globally, cardiovascular disease (CVD) is the leading cause of death. Increased low-density lipoprotein cholesterol (LDL-C) and reduced high-density lipoprotein cholesterol (HDL-C) are risk factors for atherosclerosis which increase one's risk of developing CVD. RS supplementation has been shown to improve blood lipid profiles. A meta-analysis of 20 controlled clinical studies (Yuan et al., 2018) ($n = 820$) noted that RS supplementation had a lowering effect on total cholesterol (TC) and LDL (TC mean difference, −7.33 mg/dl [95% CI 12.15 to −2.52 mg/dl]; LDL-C: mean difference: −3.40 mg/dl [95% CI – 6.74 to −0.07 mg/dl]). The average dose of RS was 16.1 g over a mean treatment period of 3.5 weeks. Overall, RS supplementation was not observed to modulate concentrations of HDL cholesterol compared to the control treatment. Yuan et al. (2018) noted a stronger lipid-lowering effect when supplementation periods were longer than 4 weeks and at higher doses of greater than 20 g/day.

Improvements in blood lipid profiles may be due to the fermentation of RS in the colon, which produces SCFAs. These SCFAs (e.g., acetate, propionate, and butyrate) are readily absorbed and concentrated in the liver; additionally, propionate may have a cholesterol inhibiting effect (Yuan et al., 2018). Mechanistic studies have noted significant reductions in TC and triglyceride concentrations, and high density lipoprotein cholesterol in streptozotocin-induced diabetic rats administered with RS2 (Zhou et al., 2015). An increased expression of genes involved in lipid metabolism pathways (including the lipid oxidation gene *Acox1*) was observed in rats administered with RS (2 g RS2 daily for 4 weeks) versus a control, with an associated decrease in genes related to fatty acid and triglyceride synthesis and metabolism (*Fads1* and *SREBP-1*) (Zhou et al., 2015). It has also been suggested that retrograded RS may bind to bile salts, enhancing their fecal excretion, stimulating the synthesis of hepatic bile acids from cholesterol, thus reducing cholesterol levels.

Yuan's (2018) meta-analysis, in addition to mechanistic studies in rodents, indicates the potentially beneficial effects of RS supplementation in improving blood lipid profiles by decreasing serum total and LDL cholesterol levels. Such findings may suggest a potential role for RS supplementation as a preventative treatment for dyslipidemia, a significant risk factor for CVD. The promising effects of RS supplementation in maintaining normal blood cholesterol levels are however not substantiated by EFSA health claims unlike the soluble fibers β-glucan (EFSA, 2010b), pectin (EFSA, 2010e), hydroxypropyl methylcellulose (HPMC) (EFSA, 2010d), and guar gum (EFSA, 2010c).

5.5 | Gut microbiome

Dietary supplementation with RS has been shown to significantly affect the gut microbiome by altering the microbial population of the human gut in both a qualitative and quantitative manner. Additionally, data from RCTs indicate that RS supplementation can increase the production of fermentation byproducts, for example, SCFAs, which may have numerous potential health benefits (Baxter et al., 2019).

Accumulating evidence suggests that the gut microbiome is a "nexus of health" (Dobranowski & Stintzi, 2021), and the alteration of its phylogenetic composition and function may offer a safe therapeutic therapy. Typically, Westernized diets are low in fermentable dietary fibers such as RS. It is reported that such low intakes may reduce bacterial diversity, lead to thinner mucus layers, compromise intestinal epithelial integrity, and increase pathogen susceptibility (Gill et al., 2021). The effectiveness of RS as a therapeutic agent may however be highly dependent on the host's baseline microbiome composition and the selected source of RS (Dobranowski & Stintzi, 2021).

RS is a microbiome-accessible carbohydrate that has been shown to affect the microbial population of the gut microbiome significantly. Most RS varieties are fermented by human gut microbiota and provide a source of carbon and energy for the bacterial species present and may thus alter the composition and metabolic activities of the gut microbiota (Fuentes-Zaragoza et al., 2011). Interindividual differences in microbiome composition are driven by genetics (e.g., host amylase gene copy number (Dobranowski & Stintzi, 2021)), environmental, and stochastic factors such as diet, drugs, anthropometric measurements, and colonization history (Valdes et al., 2018) and may play a substantial role in determining the outcomes of RS consumption.

The colon consists of a complex eco-system of microbes in which primary degraders, secondary degraders, and cross-feeders are capable of growing on RS and utilizing its byproducts to achieve a thermodynamically favorable fermentation (Dobranowski & Stintzi, 2021). The bacterial species *Ruminococcus bromi* and *Bifidobacterium adolescentis* (and other select *Bifidobacterium* species (e.g., *Bifidobacterium longum*, *Bifidobacterium fecal* (Baxter et al., 2019), and *Bifidobacterium pseudocatenulatum* (Dobranowski & Stintzi, 2021)) have been identified as human gut microorganisms with RS degrading capabilities (DeMartino & Cockburn, 2020). At present, *R. bromi* and *B. adolescentis* are the most extensively characterized primary degraders (Dobranowski & Stintzi, 2021). Such primary degraders are capable of liberating oligosaccharides from RS and producing metabolites such as lactate and acetate (Dobranowski & Stintzi, 2021).

Secondary degraders such as *Eubacterium rectale* and other butyrogenic species belonging to the *Roseburia* and *Butyrivibrio* genera, possess extracellular amylases (present in complex multienzyme systems known as amylosomes (Mukhopadhya et al., 2018)) capable of degrading starch byproducts (Dobranowski & Stintzi, 2021). In vitro studies suggest that combinations of primary degraders and secondary bacteria can produce butyrate from RS by cross-feeding (Baxter et al., 2019). For example, while *Bifidobacterium thetaiotaomicron* does not produce butyrate directly; its metabolites (lactate, acetate, and propionate) enable butyrate production by other bacteria via cross-feeding interactions (Dobranowski & Stintzi, 2021).

Different types of RS have been shown to alter the gut microbiota composition variably. Baxter et al. (2019) noted that supplementation with resistant potato starch (RPS) (RS2) increased the presence of *B. fecal/adolescentis/stercoris* sequences by 6.5-fold, while supplementation with resistant maize starch (RMS) (RS2) led to a 2.5-fold increase in the abundance of *R. Bromi* sequences. Additionally, a double-blind cross-over trial ($n = 10$) in which subjects were supplemented with either RS2 or RS4 fortified crackers (Martínez et al., 2010) noted that RS4 supplementation significantly increased Actinobacteria and Bacteroidetes while decreasing Firmicutes. Concerning species, RS4 supplementation led to increases in *B. adolescentis* and *Parabacteroides distasonis*, a bacterial population that may beneficially affect lipid metabolism disorders (Jiang et al., 2020).

In agreement with Baxter et al. (2019) and several other RS2 intervention trials, Bendiks et al. (2020) and Martínez et al. (2010) noted significant increases in *R. Bromi* and *E. rectale* with RS2 supplementation. *Ruminococcus bromi* can beneficially affect other microbial species by releasing sugars and acetate to cross-feed other species, similarly to the way in which *B. adolescentis* releases lactate and sugars (DeMartino & Cockburn, 2020).

A lack of microbial diversity and density in the human gut microbiome is commonly observed in Western societies in which high rates of chronic disease, allergies, and autoimmune disorders prevail (Valdes et al., 2018). The repopulation and diversification of the gut microbiome to more closely mimic that of our ancestors and other nonindustrialized societies may offer a potential safe therapeutic effect to promote colonic health (by improving epithelial intestinal integrity and decreasing pathogen susceptibility) and overall well-being (Jew et al., 2009; Krumbeck et al., 2018; Martínez et al., 2015).

An expert panel formed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) (2017) has defined a prebiotic as a substrate that is selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). RSs that can fulfill the criteria as a prebiotic may be included in the class of dietary carbohydrates that are resistant to degradation in the small intestine but may be later metabolized by microbes in the colon where they are fermented into SCFAs (e.g., acetate, propionate, and butyrate) and other products which may affect the health of the host (Maier et al., 2017).

Microbial fermentation products such as the SCFAs, butyrate, acetate, and propionate have several proposed health benefits, including providing energy for colonic epithelial cells, reducing inflammation, lowering risk of colon cancer, improving insulin sensitivity and the integrity of the gut barrier (Maier et al., 2017). See Figure 1 for an illustration of how RS is fermented in the colon to produce metabolites (e.g., SCFAs) with potential health benefits.

A 2-week intervention trial of 174 healthy adults who consumed either 28 to 34 g/day of RPS (RS2) or 20 to 24 g/day of resistant corn starch (RCS) (Hi-Maize, RS2) noted a significant (32%) increase in the fecal concentration of total SCFAs in those supplemented with RPS (Baxter et al., 2019). Additionally, RPS increased fecal butyrate concentrations by an average of 29% and acetate by an average of 21%; however, there was a high degree of interperson variation (Baxter et al., 2019).

It has been observed that RS supplementation may increase butyrate production in one individual but reduce it in another (Dobranowski & Stintzi, 2021), the effects of which may be greatly dependent on the individual's baseline microbiome composition. In contrast, the trial by Baxter et al. (2019) did not note any significant differences in SCFA concentrations between the control group and those supplemented with RCS (Hi-Maize). This trial highlights the significance of the origin of RS source, for example, potato versus maize RS, as well as the

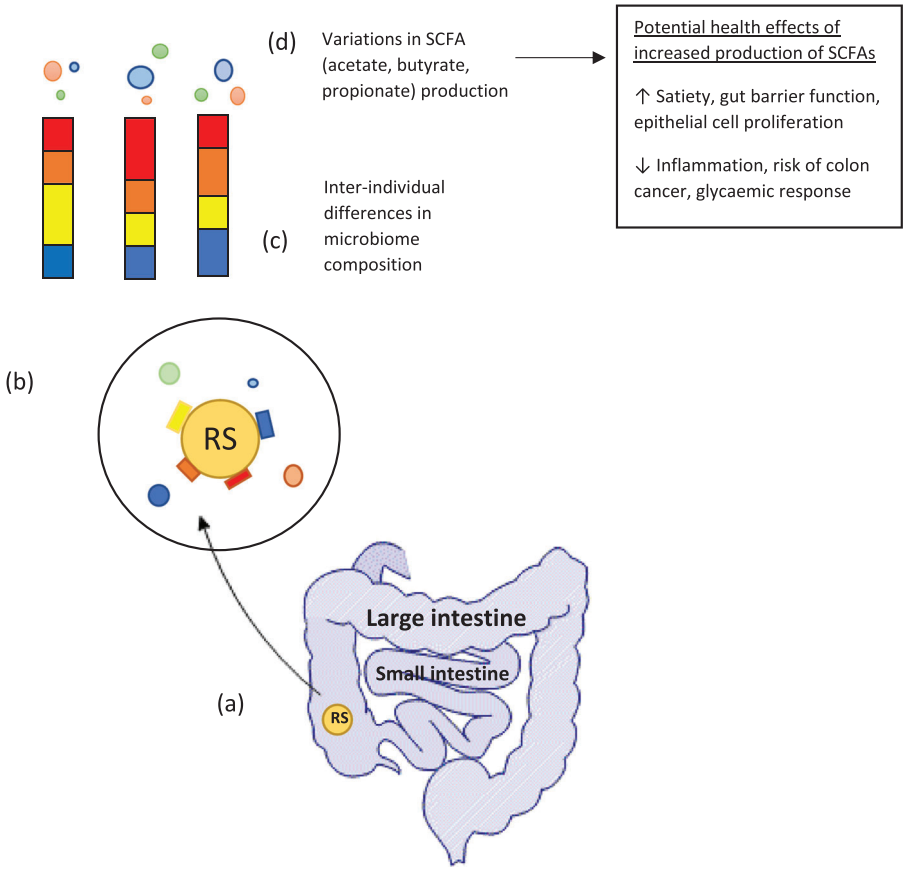


FIGURE 1 Overview of how resistant starch (RS) consumption can contribute to potential health benefits based on an individual's gut microbiome composition. (a) RS is not digested in the small intestine and enters the large intestine intact. (b) In the large intestine, RS is fermented by the human gut microbiota (represented by rectangular shapes surrounding RS) to produce metabolites including short-chain fatty acids (SCFAs; e.g., butyrate, propionate, acetate) (represented by circular shapes). (c) The potential interindividual variation in the human gut microbiome (represented by the multicolored blocks) can impact the presence and proportions of RS fermentation end-products. (d) The fermentation end products, for example, the SCFAs, butyrate, propionate and acetate (the quantity of which produced will vary by individual) have many potential health benefits

quantity/concentration of RS consumed on the effects on the individual's gut microbiome.

Furthermore, chemically modified starches with subtle structural differences may induce highly specific effects on the gut microbiome and alter fecal microbiota composition and function. Deehan et al. (2020) performed a double-blind, dose-response RCT with three types of RS4s (maize, potato, or tapioca derived) versus a placebo (digestible corn starch) in 40 healthy volunteers. Trial duration was 4 weeks and monitored subjects whose starch intake was gradually increased weekly by 10, 20, 35, and 50 g/day versus the corresponding amount of placebo. It was observed that maize- and tapioca-based RS4 sources induced an increase in gastrointestinal (GI) tolerability scores (flatulence, bloating, abdominal pain, and diarrhea) in doses >35 g/day, the effect of which was not observed in participants supplemented with potato-derived RS4 (Deehan et al., 2020).

Additionally, potato-derived RS4 was the only RS to affect bowel habits and induce a laxative effect significantly. Maize and tapioca RS4 supplemented groups showed changes in the relative abundance of bacterial taxa, the impact of which was not observed in potato RS4 or placebo supplemented groups. Although Deehan's study (2020) failed to note an alteration in total SCFA concentrations, variations in the abundance of individual SCFAs were found to be dependent on RS4 type. Maize RS4 was shown to significantly increase butyrate concentrations while tapioca RS4 increased propionate concentrations relative to baseline. The effects of supplementation were shown to plateau at a dose of 35 g RS4/day (Deehan et al., 2020).

Human intervention trials that supplement the diet with high levels of RS should aim to minimize the potential for adverse gastrointestinal symptoms. A meta-analysis of 13 RS supplementation trials (RS intakes 15–66 g/day)

($n = 428$) reported adverse effects in five trials including flatulence, abdominal discomfort, diarrhea and swelling, fullness, nausea, and constipation (Wang et al., 2019). Most of the reported symptoms were mild and disappeared after a few days of consumption. Overall, the potential adverse effects of RS supplementation in humans are like other nonstarch polysaccharides (NSPs). Overconsumption of foods high in NSPs may lead to decreased mineral bioavailability and gastrointestinal distress (Goldring, 2004).

Current evidence suggests that the effects of RS supplementation on the human gut microbiome are variable with RS type, origin, dose, and the subject's baseline microbiome composition. Future research and systematic reviews may aim to establish the mode of supplementation capable of manipulating specific bacterial populations and fermentation products in the human gut microbiome for desired health benefits. RS may however be less effective at stool bulking and improving laxation than insoluble, nonfermentable fibers such as cellulose which has a protective effect against diverticular disease (Sakamoto et al., 1996). Thus, RS supplementation as part of a diet rich in whole grains, fruits, and vegetables may be the most effective method to promote colonic health.

Overall, there is definite emerging evidence of numerous health benefits associated with RS consumption and supplementation. The most researched health effect supported by an EFSA health claim highlights the beneficial effect of RS supplementation in reducing postprandial glycemic responses and improving glucose metabolism. In addition, there is positive evidence of a potential role of RS supplementation in appetite regulation, the maintenance of normal blood lipid profiles, and the alteration of the gut microbiome composition. Although further studies are essential to determine the specific dose-response relationship between different RS types and their potential health benefits, the incorporation of high RS ingredients into commonplace food items (such as baked goods) may act as functional foods offering safe primary preventative treatments for numerous chronic diseases which prevail in Western societies.

6 | APPLICATIONS OF RS

RSs have unique sensory properties, including their fine particle size, relatively bland flavor, and white appearance. Additionally, RSs have distinct physicochemical characteristics, including increased viscosity, gel formation, swelling index, and water holding capacity, making them desirable components for many potential food products (Ashwar et al., 2016). Staple foods such as bread and pasta may be enriched with ingredients high in RS as well as food products such as those designed for special dietary requirements such as gluten sensitivity, celiac disease, and ulcerative colitis (Brown, 2004).

6.1 | RS product applications and challenges

6.1.1 | Bread

Many cereal-based foods have been reformulated to include RS ingredients and are currently on the market, including bread, pasta, tortillas, cakes, and snacks (see Table 5). Yeo and Seib (2009) investigated the effect of replacing flour with cross-linked wheat-based RS4 in white pan bread. The RS4 ingredient was incorporated at a 10%–50% flour replacement (with additional vital wheat gluten and yeast to make up for protein dilution) and had a <15% loss of loaf volume (Yeo & Seib, 2009). The firmness of the bread was shown to increase (bread with a 30% RS replacement had a crumb with 53% higher firmness value after 1 day).

A similar study (Miller & Bianchi, 2017) noted that replacement of 5%, 10%, and 15% RS4 resistant wheat starch with bread flour did not affect mixograph water absorption in white bread dough; however, a 2% increase in absorption occurred with 20 and 25% RS4 additions (Miller & Bianchi, 2017). The mixograph time was observed to increase by 15 s with the addition of 5%, 10%, and 15% RS4, by 30 s with a 20% RS replacement of wheat flour, and by 45 with a 25% RS4 replacement. Although no difference in farinograph absorption of dough at all replacement levels was observed, farinograph mixing time was shown to increase at addition levels up to 15% and decreased upon further additions. Dough strength and extensibility were not significantly affected by RS4 addition, and bread volume was also not affected until a 20% RS4 addition.

The technological difficulties associated with the reformulation of bread dough with high levels of RS ingredients have been described by Roman and Martinez (2019). Bread formulated with increasing levels of RS2 and RS3 sources has been shown to experience adverse effects on bread volume, hardness, cohesiveness, and crust color. Roman and Martinez's (2019) review suggests that to maintain bread quality, a maximum replacement of wheat flour by RS ingredients of 20% is advised.

Crusts of a paler color are commonly observed with increasing RS additions which may be attributed to the white color of starch and the lower protein content available to contribute to maillard browning; crumb color appears, however, to be less affected (Roman & Martinez, 2019). RS may dilute the function of gluten by interweaving with gluten and may also deplete the moisture in dough by gelatinization (Tian & Sun, 2020). For example, the

TABLE 5 Average nutritional composition (mean, median, and standard deviation) of resistant starch (RS)-containing cereal-based products

Product	Energy (kcal)	Total carbohydrates (g)/100 g	Sugar (g/100 g)	Fiber (g/100 g)	Protein (g/100 g)	Total fat (g/100 g)	Saturated fat (g/100 g)	Sodium (g/100 g)
Bread ($n = 26$)	188.0 ± 50.5	37.3 ± 10.0	1.8 ± 1.7	20.9 ± 11.4	13.7 ± 7.0	5.8 ± 4.5	1.4 ± 1.7	0.4 ± 0.1
Bread (median values)	160.7	39.9	2.1	24.7	10.9	3.75	0.2	0.4
Pasta ($n = 9$)	255.0 ± 74.3	74.7 ± 2.0	2.4 ± 2.5	27.5 ± 16.3	11.8 ± 1.3	1.8 ± 0.1	0.0 ± 0.0	0.2 ± 0.3
Pasta (median values)	232.1	73.2	1.8	33.9	11.8	1.8	0.0	0.1
Burger buns ($n = 10$)	156.5 ± 50.7	38.4 ± 9.1	1.1 ± 1.8	26.8 ± 8.8	15.0 ± 6.7	5.0 ± 4.2	2.4 ± 4.8	0.4 ± 0.1
Burger buns (median values)	158.7	40	0.0	24.7	13.7	3.8	0.4	0.4
Pizza crust ($n = 1$)	158.7	24.7	0.0	24.7	24.7	7.1	3.5	0.2
English muffins ($n = 1$)	140.4	42.1	0	15.8	7	0.9	0	0.3
Tortillas ($n = 17$)	172.3 ± 68.5	35.9 ± 10.4	0.2 ± 0.7	28.1 ± 6.8	12.3 ± 3.7	6.4 ± 3.1	2.0 ± 1.9	0.6 ±0.2
Tortillas (median values)	154.9	39.5	0.0	27.8	11.6	7..0	2.3	0.7
Bagels ($n = 5$)	179.3 ± 58.8	29.4 ± 7.0	0.5 ± 1.1	26.9 ± 4.0	22.1 ± 4.0	8.4 ± 4.6	2.7 ± 1.2	0.4 ± 0.2
Bagels (median values)	157.9	24.7	0.0	24.6	24.5	7.0	3.5	0.3
Sweet baked goods ($n = 3$)	189.4 ± 21.6 178.6	44.9 ± 14.2	6.5 ± 2.7	26.0 ± 10.1	11.2 ± 5.8	8.0 ± 3.9	2.7 ± 0.9	0.5 ± 0.0
Sweet baked goods (median values)	157.9	52.6	7.1	31.6	8.8	6.1	2.6	0.5

addition of high amylose maize starch to bread dough has been shown to have a gluten diluting effect and produce dough with suboptimal rheological properties, baking performance, and texture (Altuna et al., 2016).

In addition to increasing vital wheat gluten and yeast addition levels to make up for protein dilution, as described by Yeo and Seib (2009), enzymatic additives may be incorporated into cereal products to minimize gluten diluting effects and improve baking performance. Altuna et al. (2016) examined the effect of adding three enzymes; transglutamase (TG) (0–8 mg/100 g), glucose oxidase (Gox) (0–5 mg/100 g), and fungal xylanase (HE) (0–1 mg/100 g) to bread dough enriched with a high content of RS (12.5 g/100 g).

The optimum formulation of RS-enriched dough and enzymes (4 mg/100 g of TG, 2.5 mg/100 g of Gox, and 0.5 mg/100 g of HE) produced a dough with similar stickiness, work of adhesion, cohesiveness, hardness, resilience, resistance to extension and extensibility to control nonenriched dough. Compared to the regular dough, the partial substitution of wheat flour (WF) with RS led to a higher crumb firmness and lighter crust. Although the partial substitution of WF with RS was shown to delay the staling process of the bread loaf, the further addition of TG, Gox, and HE accelerated this process compared to the regular dough.

Similarly, Arp et al. (2021) examined the effect of adding two modified celluloses (MC), HPMC, and carboxymethylcellulose to bread dough enriched with 30% RS2 (maize starch). Arp et al. (2021) observed improvements in the technological quality of the dough upon addition of both MCs as noted by a highly cross-linked, well-developed gluten network with a superior baking performance than non-MC enriched dough with added RS. A significant increase in farinographic water absorption was also noted in MC added doughs, resulting in higher water content and improved viscoelastic properties. The positive effects reported by MC additives may contribute to enhanced dough quality and expansion during the leavening and baking steps of high RS bread doughs.

6.1.2 | Pasta

Current research suggests that incorporating RS ingredients into pasta formulations may reduce in vitro starch digestibility and enhance the nutritional value of conventional pasta. Aravind et al. (2013) evaluated the effects of enriching pasta with two commercially available RSs (RS2, Hi Maize^{TM} 1043 and RS3, Novelose 330^{TM}) at 10%, 20%, and 50% substitution of durum wheat semolina for RS2 and 10 and 20% substitution of RS3. Aravind et al. (2013) observed that 10 and 20% RS2 and RS3 substitutions of semolina did not significantly affect pasta cooking loss, texture, and sensory properties, though led to a slight decrease in uncooked pasta yellowness. Additionally, both commercial RS2 and RS3 were shown to lower in vitro starch hydrolysis than control pasta (100% durum wheat) (Aravind et al., 2013). RS-enriched pasta showed lower enzymatic digestibility noted by smaller AUC values in comparison to control pasta. These effects were likely due to the replacement of RDS in pasta with RS.

In agreement with this study, Bustos et al. (2011) noted a lower in vitro predicted glycemic index (PGI) with the addition of RS4 and RS2 to pasta formulations at 7.5 and 10 g/100 g in comparison to a control pasta (PGI of control pasta was 83.3 ± 0.3, in comparison to RS2 enriched pasta 7.5 g/100 g and 10 g/100 g which had a PGI of 66.2 ± 1.0 and 66.4 ± 0.6, respectively, and RS4-enriched pasta 7.5 g/100 g and 10 g/100 g with PGI values of 70 ± 2.5 and 65.5 ± 1, respectively).

In comparison to conventional spaghetti and spaghetti enriched with 10% RS3 or bran, Sozer et al. (2007) found that the hardness and adhesiveness values of bran spaghetti were higher than control and RS spaghetti, while the hardness values of control and RS-enriched spaghetti were similar. These results may highlight the benefits of RS-enriched pasta versus other high-fiber spaghetti on the market, such as spaghetti enriched with bran fiber. Although both are rich sources of dietary fiber, RS-enriched spaghetti was less sticky, had lower cooking losses, and had similar firmness values to the control (Sozer et al., 2007) which may increase consumer acceptability of fiber-enriched spaghetti.

6.1.3 | Tortillas

Wheat tortillas have significantly increased in popularity in Western diets, and the addition of RS may help to improve their nutritional profile without negatively influencing their sensory attributes. Alviola et al. (2012) investigated the effects of substituting commercial tortilla and bread flours with cross-linked RS4 at 0%, 15%, 20%, and 25%. Alviola et al. (2012) noted that RS4 substitution decreased mixing resistance of tortilla and bread flour dough without significantly affecting water absorption. Additionally, the RS4 substituted tortillas required significantly less force to extend (4.5–8.8 N) versus the control tortillas (8.7–10.6 N), indicating that they were softer in texture. It was observed that the RS4-substituted bread flour tortillas were significantly more shelf-stable and maintained good flexibility post 18 days of storage in comparison to RS4-substituted tortilla flour-based tortillas which lost flexibility after 4 days of storage (Alviola et al., 2012).

6.1.4 | Gluten-free food products

Gluten-free products are commonly regarded as being of suboptimal nutritional quality and often have lower levels of dietary fiber and RS, as well as a higher glycemic index, compared to their gluten-containing counterparts (Giuberti & Gallo, 2018). Thus, the incorporation of RS-rich ingredients may help to improve the nutritional quality of gluten-free diets.

Foschia et al. (2017) successfully incorporated a RS2 HAMS as a fiber enriching ingredient (100–200 g/kg) into gluten-free pasta (Foschia et al., 2017). The addition of RS was shown to improve the pasta quality compared to a control sample by increasing the firmness and decreasing cooking loss and stickiness value. Additionally, studies including Giuberti et al. (2017) and Sarawong et al. (2014) have demonstrated the successful incorporation of green plantain flour and resistant native waxy rice starch into gluten-free bread and rice cookies, respectively. The addition of RS to gluten-free products may be beneficial to consumers with celiac disease by improving the nutritional quality to help lower the risk of chronic degenerative diseases (Foschia et al., 2017).

7 | ACCEPTABILITY OF RS-ENRICHED FOODS

Reported barriers to dietary fiber consumption include the inherent characteristics of fiber, time pressures, cost, and limited availability of fiber-rich foods (Mohr et al., 2010). Foods high in fiber are typically perceived to be coarser, denser, and often less palatable than refined, processed foods. Potential solutions to increasing population fiber levels include changing the qualities of dietary fiber including taste, texture, and general aesthetics (Mohr et al., 2010). Due to the unique sensory properties of RSs (fine particle size, bland flavor, and white appearance) in comparison to other sources of dietary fiber (e.g., brans and gums), they may offer greater consumer acceptability of high-fiber products to those accustomed to a refined Western-style diet (Sajilata & Singhal, 2005).

7.1 | Sensory studies

Sensory data have demonstrated that RSs can replace significant proportions of conventional flours (high in digestible carbohydrates) in staple cereal-based products without affecting overall liking or acceptability. Miller and Bianchi (2017) reported that RS-enriched white pan bread did not significantly differ in liking of flavor, texture, or overall liking in bread at replacement levels of 15%, 20%, and 25% conventional flour (by an untrained panel of 97 consumers). Similarly, Bustos et al. (2011) noted that pasta formulations containing 7.5 and 10 g/100 g of RS4 and RS2 did not affect overall acceptability in comparison to control pasta. Furthermore, Alviola et al. (2012) noted that tortillas formulated with 15% Fibersym RW (RS4) (flour basis) had a higher overall acceptability than the control.

Sweet baked goods including muffins and biscuits may also be reformulated with RS ingredients with high acceptability. Maziarz et al. (2013) investigated the sensory characteristics of high-amylose maize (HAMS-RS2)-enriched muffins (5.50 g RS/100 g) using a nine-point hedonic scale. The RS-enriched muffin was perceived as significantly moister than the control muffin (baked using all-purpose flour) by the sensory panel ($n = 37$). Additionally, the muffins scored significantly higher for color, mouthfeel, density, and had a higher overall mean likeability score versus the control.

Similar results were noted by Baixauli et al. (2008a) who reported that replacing wheat flour with an RS2 ingredient (Hi-Maize 260) at levels of 0%, 5%, 15 and 20% did not significantly affect the taste, overall acceptance, and consumption intention of the muffins ($p < .05$). Baixauli et al. (2008a) did however note significant differences in the appearance and texture of the muffins. The cohesiveness, typical taste and odor, number of gas cells, and springiness and chewiness attribute scores decreased upon the addition of RS. As observed in Maziarz et al.'s (2013), the moisture content of the muffins increased in RS-enriched muffins; additionally, the sweetness perception increased despite an equal concentration of sugar present across the formulations. Furthermore, panelists noted a sensation of grittiness in RS-enriched muffins which was not present in nonenriched muffins (Baixauli et al., 2008a)

Laguna et al. (2011) investigated the effects of replacing wheat flour with 20, 40, or 60 g/100 g RS2 (Hi-Maize 260) on the baking and eating quality of short-dough biscuits in comparison to control biscuits. RS2 addition increased the breaking strength, crumbliness, and paleness of the surface and crumb while reducing the resistance to penetration of the biscuits. While the sensory acceptance of the RS2 enriched biscuits with 20 g/100 g did not differ signficantly from the control, higher levels of 40 g/100 g reduced the acceptability of the color, appearance, and texture without altering the taste, sweetness, and overall acceptance or consumption intention (Laguna et al., 2011). Lower ratings for sensory acceptability and reduced consumption intention were however observed at higher doses of RS2 (60 g/100 g).

Additionally, RS may be incorporated into snack foods commonly consumed in Western societies. Aigster et al.

(2011) evaluated the effects of adding two levels of RS (10 g/100 g or 15 g/100 g) versus a control (0 g RS/100 g) on the physicochemical and sensory properties of granola bars. The addition of RS resulted in bars of a lighter color, which scored lower for sweetness, crunchiness, and moisture. It was observed that there was no significant difference between the mean acceptability scores between the controls and supplemented granola bars.

7.2 | Consumer perception of RS

A nationwide postal Food and Health Survey ($n = 849$) (Mohr et al., 2010) conducted among Australian adults investigated the impact of engagement with the health benefits of dietary fiber and receptiveness to resistant starch. In total, 15.7% of participants reported that they were aware of the health benefits of RS. The study noted that women were more "fiber-engaged" than men and were, in addition, more receptive to RS and its potential health benefits than men. Additionally, fiber engagement was significantly predicted by increasing age, education level, and being female, though not by income.

When rating the acceptability of foods as a means of delivering RS, participants noted a preference for food staples and those with existing asscociations with fiber (e.g., breakfast cereals, pasta, noodles) over indulgent food options (such as white bread, sweet, and snack foods). Women showed a greater disapproval of delivering RS via indulgences and the margin between acceptability of staples over indulgences was noted to increase with an increase in fiber engagement (Mohr et al., 2010).

Baixauli et al. (2008b) (investigated the impact of information about the fiber content of plain, wholemeal, and RS-enriched muffins on consumer acceptability ($n = 102$). With and without label information, the plain muffins received similar ratings. In contrast, in the absence of label information on fiber content, wholemeal muffins received a low acceptability score which increased when the participant received nutritional information. Although a slight increase in acceptability score was observed when information was provided for the RS-enriched muffin, the rise in acceptability score was lower than that observed for the wholemeal muffins (though the overall acceptability of RS-enriched muffins was still higher), despite the muffins reporting identical fiber contents. Additionally, the wholemeal muffin scored higher for the "healthy" attribute. As the RS-enriched muffin was more similar in appearance to the plain muffin, consumers may not have believed that the fiber content is accurate or acts in the same way as conventional fibers (e.g., bran) or may have perceived it as being somehow unnatural (Baixauli et al., 2008b).

Current research thus highlights the successful incorporation of RS into a wide variety of food products. At appropriate levels, RS is an acceptable replacement for conventional flours. The addition of RS to food products may offer numerous potential health benefits to the consumer (including a reduction in glycemic index in comparison to their traditional counterparts), while simultaneously benefiting the manufacturer by improving the technological and/or sensory properties of a foodstuff, providing a fiber-enriched product which both looks and tastes equivalent to its nonenriched control. However, there is likely poor consumer awareness of RS and its potential health-promoting properties. Increased nutritional knowledge about RS may help to increase its acceptability and the potential marketabilty of RS-enriched foods.

8 | MARKET ANALYSIS OF RS INGREDIENTS AND PRODUCTS

8.1 | RS ingredients

Market offerings for RS ingredients are diverse and continuously expanding (see Table 4 for a descriptive summary of commercially available RS ingredients). The commercial availability of RS ingredients is advantageous as they are significantly less affected by processing and storage conditions than native sources of RS (e.g., potatoes, bananas) (Nugent, 2005). Ingredion produces a variety of commercially available RSs based on high-amylose maize starch (Hi-Maize®260), resistant tapioca starch (Novelose®3490), resistant rice starch (Novelose®8490), and modified potato starch (Versafibe1490 and Novelose®330).

There are various RS3 and RS4 fiber ingredients entering the market that can be readily incorporated into baked goods such as bread, biscuits, cakes, pasta, breakfast cereals, tortillas, and energy bars. MGP Ingredients's Fibersym®RW (MPG, 2020) is a wheat-based high RS4 ingredient that can be manufactured as gluten-free by FDA standards and is marketed as a low calorie (0.4 kcal/g) dietary fiber supplement. Additionally, Versafibe™ 1490 (a potato-based RS4), Novelose® 3490 (a tapioca-based RS4), Novelose8490 (a rice-based RS4), and Novelose330 (a potato-based RS3), all produced by Ingredion, are marketed as low FODMAP, gluten-free and low calorie, high dietary fiber flour substitutes.

An exploration of the RS market reveals a growing interest in green banana starch flours. Although bananas are high in starch and have high amylose to amylopectin ratio (Kaur et al., 2020), in their unripened state, they remain high in native starch granules rich in RS2. Commercially available green banana flours are commonly marketed as

gluten-free, flour, and sweetener options high in fiber and suitable for ketogenic diets (see Table 4).

RS supplements with marketed "prebiotic" effects are also available for commercial use. Solnul™ is a high RS2 (>60% RS2) unmodified potato starch ingredient produced by MSP Starch Products Inc. Although Solnul® is marketed to promote several positive health effects related to GI metabolic health, the FDA has currently evaluated no such claim. Products such as MS Prebiotic (MSPrebiotic Inc., 2021) include Solnul™ as a functional ingredient and are currently sold in small quantities for personal use (e.g., a supplement for smoothies).

The energy contributions of the RS ingredients were reported to range between 0.25 and 2.78 kcal/g. This high degree of variability may be attributed to the varying levels of purity of the fibers (a higher percentage of digestible carbohydrates will increase their energy contribution) and the lack of a universally agreed energy contribution of RS. According to FDA regulations, RS (an insoluble fiber) is reported in food labels as a noncaloric ingredient (0 kcal/g) in the United States (FDA, 2016), whereas in the European Union all types of fiber are assigned an energy contribution of 2 kcal/g (EFSA, 2010a).

Giles et al. (2019) have argued that the fermentation of RS can produce energy so that RS cannot be considered a noncaloric food ingredient. RS could contribute significantly to total daily energy intakes; thus, if the contribution of RS is not correctly included on food labels, daily energy intakes may be underestimated (Giles et al., 2019). The underestimation of the energy contribution of RS may have further negative implications for the design of human intervention trials. Without accounting for the energy contribution of RSs, one may confound the interpretation of energy balance studies (Giles et al., 2019).

8.2 | RS products

Speciality starches may be added to food products to act as functional ingredients and positively contribute to increased expansion, improved crispness, reduced oil pick up, and improved overall eating quality (Sajilata & Singhal, 2005). RS is typically added to food products to increase the fiber nutritional claims and lower the caloric value of food products compared to traditional carbohydrate sources. Speciality starches are increasingly replacing unmodified starches due to their robustness and capacity to endure harsh processing conditions.

A review of 72 cereal-based products (bread, pasta, burger/hot dog buns, pizza crust, English muffins, tortillas, bagels, and sweet baked goods) from four different countries (see Figure 2 for countries of origin of reviewed RS-containing products) containing RS ingredients was conducted. Most of these RS-containing products were sold in the United States ($n = 49$) and Australia ($n = 14$). Over 40% of the RS-containing tortillas were produced in Mexico. The products included in the analysis contained RS as a replacement ingredient for conventional flours. In most products, RSs were added as fiber enhancing ingredients, and all products meet the criteria for a "high fiber" nutrition claim according to EC Regulation 1924/2006 (European Commission, 2021).

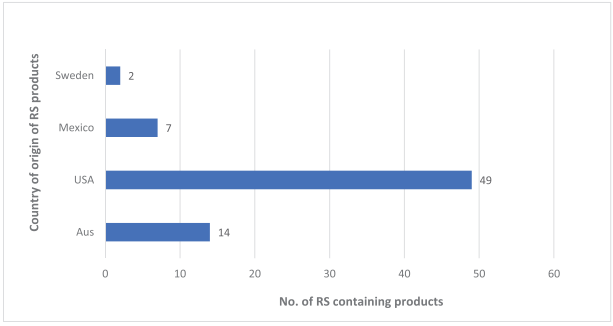


FIGURE 2 Countries of origin of reviewed resistant starch (RS)-containing products

The different types of RS ingredients (as reported on the product label) in the products included in the review are shown in Figure 3. It may be observed that modified wheat starch/resistant wheat starch was the most added RS ingredient, present in approximately three-quarters of the cereal-based products reviewed ($n = 55$), followed by tapioca-based RSs ($n = 9$) and maize-based RSs ($n = 5$), three products with RS ingredients from unspecified origins were also included (modified food starch and resistant starch).

The average nutritional composition of the RS-containing cereal-based products were evaluated and compared to average values of their control counterparts, and a total overview of nutrients per 100 g of product can be seen in Table 5 (see Table S1 for nutritional composition of control products). The nutritional composition (in g/100 g) of the RS-containing bread was evaluated against control bread (white wheat, wholemeal, and seeded). The RS-containing bread included in this review was diverse in formulation and included white, multigrain, seeded, and flavored bread (e.g., honey, cinnamon). To account for the high degree of variability in formulations, the median nutrient values in g/100 g were used to measure centrality.

It was observed that the median energy values for RS-containing bread were lower than control bread (160.7 kcal/100 g vs. 233, 223, and 253 kcal/100 g for white wheat, wholemeal, and seeded control bread, respectively). The lower energy values for RS-containing bread may be anticipated as RS ingredients are typically marketed as having significantly lower energy values (experimentally,

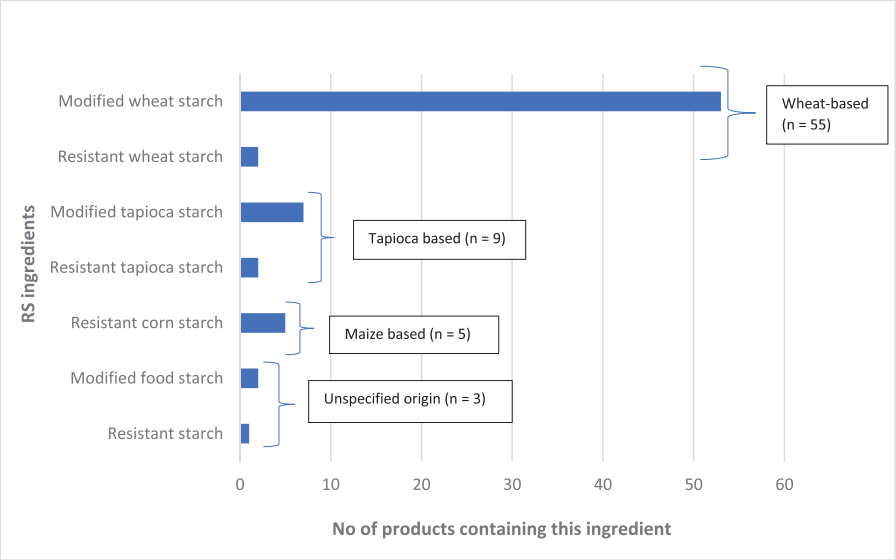


FIGURE 3 Number of different resistant starch (RS) ingredients (as reported on product lables) present in the reviewed cereal-based products

the energy value of RS is approximately 2 kcal/g (Nugent, 2005) but may be as low as 0.25 kcal/g (see Table 4) than traditional carbohydrate sources (4 kcal/g). Over three-quarters of RS-containing bread were of US origin, where RS (an insoluble fiber) is labeled as a noncaloric (0 kcal/g) ingredient (in the European Union all fibers are assigned an energy contribution of 2 kcal/g (EFSA, 2010a)) which may significantly affect the reported energy contribution on the product label.

In agreement with these findings, the median value of total carbohydrates/100 g for the RS-containing bread was lower than that of conventional bread (39.9 g/100 g for RS-containing bread vs. 44.6, 39 g, and 41 g/100 g carbohydrates for white wheat, wholemeal and seeded control bread, respectively). The lower median carbohydrate values of the RS-containing bread may also be expected as these products were often marketed to health-conscious consumers as being "low carb," "keto-friendly," "carb smart," or "light" alternatives to conventional bread and may have been formulated to have lower carbohydrate contents. RS-containing bread were also higher in protein and fat, which may have displaced the carbohydrate rich ingredients.

The fiber contents of RS-containing bread were significantly higher than control bread, having a median fiber content of 24.7 g fiber/100 g (range 7–40 g fiber/100 g) in comparison to the 3-control bread (2.8, 6.5, and 7 g fiber/100 g, respectively, for white wheat, wholemeal and seeded controls). The RS-containing bread with the lowest fiber contents (ranging between 7 and 7.5 g fiber/100 g) was marketed as white wheat bread, with soft textures that would appeal to children while adding fiber to the diet. Despite their appearance and texture, the RS-containing white wheat bread had a similar fiber content to the wholemeal and seeded control bread (8 and 7 g/100 g fiber, respectively).

Additionally, the RS-containing bread was typically higher in protein, median value 10.9 g protein/100 g, range (4.4–24.7 g/100 g vs. 8.7, 9.8, and 10.8 g protein/100 g for white wheat, wholemeal, and seeded control bread, respectively). This observation may again be attributed to the marketing of the products as being "keto-friendly" to appeal to a health-conscious consumer. Such "high protein" RS-enriched bread contained protein fortifying ingredients such as wheat protein isolate, vital wheat gluten, and egg white powder. The total fat (in g/100 g) of the RS-containing bread was also higher than that of controls (median value 3.75 g/100 g, range 1.8–18.5 g/100 g) compared to the control bread (1.4, 1.7, and 3.5 g/100 g fat for white wheat, wholemeal, and seeded control bread, respectively). The RS-containing bread that was found to be particularly high in fat was marketed as being "keto-friendly" and "low carb" bread alternatives (in addition to seeded and flavored bread).

Similar nutritional trends were noted in the other cereal-based RS products analyzed in comparison to conventional controls (see Table 5). RS-containing pasta, burger buns, tortillas, and bagels were typically lower in energy (kcal/100 g) while significantly higher in fiber. A limited selection of additional RS-containing products were noted in the market review, including pizza crust (n = 1), English muffins (n = 1), and sweet baked goods (n = 3). These products typically had strikingly high-fiber contents with median fiber contents of 24.7, 15.8, and 26 g fiber/100 g for pizza crust, English muffins, and sweet baked goods, respectively.

This market analysis of RS-containing products, although exhaustive, may have several limitations. Online access to product databases may have been limited as certain websites were not accessible from the country of research, additionally, only products which had product information available in English were included in this review. Current labeling guidelines for RS are somewhat unclear. As Nugent (2005) explained, RS may be included within the term "fiber" on the nutrition labels in some countries but not in others. In addition, RS can only be claimed as an ingredient on the nutrition label if the appropriate fiber determination method applied to that product is approved in the country of interest (Goldring, 2004).

Some products, especially gluten-free products, which often contain a high percentage of modified starches, were omitted from the analysis as they were not clearly marketed as containing RS ingredients. Therefore, certain products may have a higher fiber content than stated on the label specification if the contribution of RS is not included. Moreover, as RS4 chemically modified starches and ingredients from genetically modified organisms are not permitted to be sold within specific countries, this may further limit RS-based products' sale and market.

9 | CONCLUSION

RS is an intriguing dietary fiber source that may offer nutritional benefits such as improving glucose metabolism, blood lipid profiles, increasing satiety, and reducing dysbiosis of the gut microbiome. Typically, RS ingredients may be classified as fermentable fibers with low solubility and viscosity allowing them to be readily incorporated into a wide variety of foods. RS-enriched foods, consumed as part of a healthy diet (rich in whole grains, fruits, and vegetables) or in combination with other fiber isolates with varying physicochemical properties (e.g., nonfermentable, soluble, and viscous fibers), may help support the maintenance of a rich and healthy microbiota and reduce one's risk of developing numerous chronic diseases.

Although the replacement of traditional flours with RS ingredients may offer technical challenges concerning gluten dilution and a reduction in maillard browning, the incorporation of high RS ingredients (at the appropriate replacement level) may contribute to enhanced consumer acceptability in comparison to traditional fiber sources (e.g., bran and gums) due to their small particle size, relatively bland flavor, and white appearance. Such innovative products have the potential to appeal not only to the health-conscious consumer but to the typical Western consumer whose palate may favor processed refined grains. Greater public awareness of the benefits of RS and access to RS-enriched foods may additionally help to increase their acceptability.

Considering the evidence provided in this review, the reformulation of stable food products to contain high RS ingredients may provide a pragmatic opportunity to offer high-fiber, reduced energy, functional food products with a lower glycaemic index, increased satiety markers, and prebiotic effects in comparison to their conventional counterparts. While the presence of food products containing high RS ingredients is still quite limited (particularly in European countries), a market analysis highlights a broad range of commercially available RS ingredients which the food industry may readily exploit to develop commonplace food products which may assist consumers in increasing their dietary fiber intakes and subsequently reduce their risk of developing numerous chronic diseases which currently plague Westernized societies.

Future research in RS may focus on developing techniques to isolate and characterize RS1 and RS5 to investigate their potential health-promoting effects and applications in the food and health sectors. It is essential that universally accepted characterization techniques are established to quantify RSs both qualitatively and quantitatively, so food labeling and nutritional databases can be updated to assess population exposure to RS and its contribution to daily fiber intakes. Moreover, the establishment of an accepted energy contribution for RS is vital, current US food labeling guidelines which recognize RS as a noncaloric ingredient may be misleading. If the contribution of RS is not correctly included on food labels, daily energy intakes may be underestimated, and the interpretation of energy balance studies may be confounded.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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