\_\_\_\_Starch

### A Comprehensive Review Resistant Starch-Containing Bread as a Functional Food: Its Effect on Appetite, Glycemic Index, and Glycemic Response

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Resistant starch (RS) passes through the small intestine undigested and is fermented in the large intestine. Due to this feature, RS functions as a prebiotic and, RS is added to various foods as a functional fiber. RS for traditional fiber has various advantages, such as increased viscosity, gel form, and volume increase. In terms of these advantages, RS, which is commercially produced, is used in the food industry for bread, breakfast cereals, cooked products, and pasta products. Bread is a food that is frequently consumed in human nutrition. Although bread is a frequent food, the fiber content of the bread is low and the glycemic index (GI) of the bread is high. For this reason, bread-RS is used to convert the bread into a healthier version. Adding RS to bread increases the bread's fiber content and decreases the GI of the bread. RS reduces not only the GI of foods but also the glycemic response of individuals after consumption. RS, which can be fermented in the colon, is converted into short-chain fatty acids (SCFAs) because of fermentation. RS affects glucose and insulin levels through the production of SCFA, which increases glucose uptake in the muscles and liver, releases intestinal hormones, and increases insulin sensitivity. The addition of RS to bread results in a product with a high fiber content and a low GI.

### 1. Introduction

Consumers' increasing awareness of the relationship between diet and health has increased the popularity of new foods with nutritional properties. Within this context, the importance of functional foods that have basic nutritional functions and provide physiological benefits and/or reduce the risk of chronic diseases is increasing day by day.<sup>[1]</sup> According to the European Food Safety Authority (EFSA), functional foods positively affect one or more

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functions in the body by improving health and/or reducing the risk of disease and providing adequate nutrition. A functional food can be a natural food or food to which an ingredient has been added or from which an ingredient has been removed using technological or biotechnological means.<sup>[2]</sup> Several foods such as fermented products, seafood, whole grains, legumes, nuts, seeds, vegetables, and fruits are examples of functional foods. Functional foods can also be obtained by adding biologically active substances such as minerals, vitamins, fatty acids, dietary fiber, antioxidants, prebiotics, and probiotics to foods.<sup>[3]</sup> Low consumption of dietary fiber, which is a functional element, is associated with the risk of cancer, cardiovascular diseases, obesity, and type 2 diabetes. Evidence that dietary fiber reduces elevations in postprandial glucose levels suggests that fiber consumption should be increased for the prevention and treatment of these chronic conditions.<sup>[4]</sup> Therefore, foods such as bread, noodles, and biscuits whose consumption rate is high have been

enriched with dietary fiber in recent years. However, adding or dinary dietary fiber to foods adversely affects the food's taste, appearance, and texture. Therefore, the wide use of ordinary dietary fiber in foods has been limited and using alternative ingredients has increased. Within this context, studies on using resistant starch (RS) as a food supplement are promising.<sup>[5]</sup> In this review, the physiological effects of using RS in bread were discussed and the effects of consuming RS-containing bread on appetite, glycemic index (GI) and glycemic response were compiled.

Starch is a polymeric carbohydrate produced by plants to store energy.<sup>[6]</sup> Two main polysaccharides (amylose and amylopectin) are the basic building blocks of the structure of starch. Both amylose and amylopectin linear glucose chains are linked by  $\alpha$  (1  $\rightarrow$  4) linkages. With its branched chain structure, amylopectin forms a complex structure with  $\alpha$  (1  $\rightarrow$  6) linkages.<sup>[7]</sup> Amylose constitutes 15%–20% of starch. Two basic crystalline starch structures, A and B types, have been defined. Type A starches are found in grains, while type B starches are found in tubers and foods rich in amylose. Type A and B starches contain different proportions of amylopectin. A third type, also called type C, is found in legumes as a mixture of forms A and

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Table 1. The	average	resistant	starch	consumption	amounts	of various
countries.						

 Table 2. Resistant starch amount of commonly consumed starchy foods.

Country	Average resistant starch consumption [g day <sup>-1</sup> ]
Australasia	
Australia	5.15 g (3.4–9.4 g range
New Zealand	5.7 g
Europe	
Europe (mean)	4.11 g
Belgium	3.99 g
Denmark	3.67 g
England	3.97 g
France	3.73 g
Germany	3.75 g
Italy	8.50 g (7.2 g north-west 9.2 g south)
Netherlands	5.29 g
Norway	3.22 g
Spain	5.74 g
Sweden	3.36 g
Switzerland	4.38 g
United Kingdom	2.76 g
North America	
United States of America	4.9 g (2.8–7.9 g range)
Asia	
China	14.90 g
India	10.00 g
Africa	
Africa countries	35.00 g

Foods	Resistant starch amount [g/100 g]
Cereals and cereal products	
Bread (white)	1.2
Bread (whole wheat)	1.0
Oats (uncooked)	11.3
Barley (grained and cooked)	2.4
Millet (cooked)	1.7
Rice (brown and cooked)	1.7
Rice (white and cooked)	1.2
Pasta (cooked)	1.1
Pasta (whole grain)	1.7
Cornflakes	3.2
Muesli	3.3
Fruit and vegetables	
Pea (cooked)	1.9
Potatoes (boiled)	1.3
Potatoes (cooked)	1.0
Sweet Potatoes (baked)	0.7
Fried potatoes	2.8
Corn (cooked)	0.3
Yams	1.5
Banana (raw)	4.0
Banana (plantain/cooked)	3.5
Legumes	
Beans (white, canned/cooked)	4.2
Mung beans (canned/cooked)	1.6
Chickpeas (canned/cooked)	2.6
Lentil	3.4

B.<sup>[8]</sup> Given the nutritional properties of starch, it is divided into three main classes: rapidly digestible starch, slowly digestible starch, and RS. While rapidly digestible starch is hydrolyzed to dextrins by the  $\alpha$ -amylase enzyme within 20 min after it is consumed, slowly digestible starch is hydrolyzed within 120 min. On the other hand, RS is a type of starch not hydrolyzed in the small intestine of healthy individuals but fermented in the large intestine.<sup>[9]</sup> RS is physically or chemically resistant to  $\alpha$ amylase digestive enzymes in the upper gastrointestinal tract and is fermented in the large intestine. RS contains  $\alpha$ -linked glucose molecules resistant to hydrolysis in the small intestine.<sup>[6]</sup> Adding RS to various foods leads to the development of new functional foods.<sup>[5]</sup>

In a study conducted to determine the daily RS intake of individuals, the daily RS intake of Americans was approximately 4.9 g day<sup>-1</sup> (ranging between 2.8 and 7.9 g day<sup>-1</sup>).<sup>[10]</sup> The amount of daily intake of RS is thought to be 3–6 g day<sup>-1</sup> in Europe, and 5–7 g day<sup>-1</sup> in Australia.<sup>[11]</sup> The average RS consumption amounts of various countries are shown in **Table 1**.<sup>[12–14]</sup> The data analysis obtained from the published literature showed that the content of RS (>3 g/100 g cooked portion) was high in fried Italian bread, pumpkin bread, corn flakes, muesli, wheat flakes, rice flakes, potato chips, raw bananas, white beans, and lentils. There is no standard database on the RS content of commonly consumed foods. Methods for measuring the amount of RS in foods are also not standardized.<sup>[15]</sup> Factors such as the physical form of grains and seeds, the relationship between starch and other dietary components, and the size and type of starch granules affect the RS content of foods. Food preparation processes such as cooking, and grinding can affect the amount of RS in foods.<sup>[16]</sup> It is also possible to increase the RS content in foods by changing some processing conditions such as pH, duration and temperature of heating, the number of heating and cooling cycles, and freezing and drying.<sup>[17]</sup> According to the data in the literature, the RS content of commonly consumed starchy foods is shown in **Table 2.**<sup>[18]</sup>

Starch is included in the composition of many foods and gains foods' important functional properties. Because RS has a sweet, white particle whose size affects the texture of the product at the maximum level, its use in different foods provides an advantage. The use of RS in foods has such positive effects as increasing viscosity, forming a gel, increasing the crunchiness of coated products, increasing the crunchiness of breakfast cereals, being used as a functional ingredient, and providing lower water holding capacity than conventional fiber products. Due to its functional properties, RS is used in the food industry in products produced for celiac patients, low-carbohydrate commercial foods, fermented foods (sausage, etc.), bread, cakes and

Table 3. Types of resistant starch, definitions, and foo	ood samples.
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Resistant starch type	Definition	Food sample
Type I resistant starch	Physically inaccessible starch	Milled grain or seeds, legumes
Type II resistant starch	Granular starch containing B- or C-polymorphs	High amylose corn, raw potato, and raw banana starch
Type III resistant starch	Retrograded starch	Cooked and chilled foods (potatoes, pasta, rice)
Type IV resistant starch	Chemically modified starch	Cross-linked starch and octenyl succinate starch
Type V resistant starch	Amylose–lipid complex	High amylose starch with stearic acid complex

breakfast cereals, baked goods, pasta products, and products produced for patients with diabetes.  $^{[19]}$ 

### 2. Resistant Starch Types

In foods, there are five types of RS called type I, II, III, IV, or V RS according to its structure.<sup>[20]</sup> RS types, definitions, and food samples are given in **Table 3**.<sup>[18,20]</sup>

### 2.1. Type I Resistant Starch

Type I RS is synthesized in the endosperm of cereal grains or seeds and occurs originally in nature. Among the examples of foods containing type I RS are legumes, coarsely ground grains, and bread and pasta made from these grains. Starch granules are surrounded by protein matrix and cell wall. This physical structure prevents starch from being digested and reduces the glycemic response to be produced by starch.<sup>[18]</sup> Other components within the cell, such as protein and lipid bodies, cytoplasmic matrices, and enzyme inhibitors, create an extra barrier to enzyme hydrolysis. In type I RS, especially the protein matrix, the starch is resistant to digestion. In the formation of type I RS, starch granules with proteins cause a slow reaction of digestive enzymes, and lower starch gelatinization occurs by preventing water diffusion.<sup>[21]</sup> As a result of enriching bread with type I RS, the amount of RS in the bread increased, and the starch digestibility of the bread decreased. RSs used in bread have been commercially produced or isolated from coarse grains (whole grain rye, barley, flaxseed, and whole grain) and legumes (peas, boiled white beans, and germinated mung beans).<sup>[21,22]</sup> The resistance to the digestion of type I RS can be reduced by milling or chewing. In food processing, which includes heat and moisture, generally, type I RS is destroyed.<sup>[23]</sup>

### 2.2. Type II Resistant Starch

Uncooked potato starch, green banana starch, ginkgo starch, and high amylose cornstarch are examples of type II RS. Type II RS is originally found in nature, as is type I RS. However, after potatoes and bananas are cooked, most of the starch in them becomes digestible due to gelatinization and loss of type B and C crystals.<sup>[18]</sup> High amylose corn starch consists of small-sized granules. It also has low water retention and high-water binding properties. High amylose cornstarch can be added to foods to increase total dietary fiber intake without significantly altering the physicochemical properties of foods.<sup>[24]</sup> The resistance to the digestion of type II RS is generally higher than that of type I RS. The resistance of type II RS to digestion can be reduced by food processing and cooking, which include heat and moisture.<sup>[23]</sup> Various components affect the amount of type II RS found in foods. The addition of simple sugars (glucose, sucrose, maltose, and ribose) increases the RS content of high amylose corn starch, while the addition of various fibers such as cellulose, lignin, and pectin in potato starch prevents the formation of RS.<sup>[9]</sup> The use of commercial type II RS in bread dough led to increased water absorption, softening degree, and elongation resistance. The use of commercial type II RS in bread at a rate of 20% resulted in an increase in dietary fiber, and a decrease in hardness, crumb texture, and porosity. The use of 30% type II RS in bread led to a decrease in specific volume and an increase in hardness and crumb density.<sup>[25]</sup>

### 2.3. Type III Resistant Starch

Type III RS is retrograde amylose and retrograde starch. It is produced from natural starch by a three-step process. The first step, called gelatinization, involves the degradation of the granular structure by heating it with excess water. The second step, called retro-gradation, involves the slow recrystallization of amylose molecules after the cooling or dehydration of the starch solution. Finally, the resistant fraction can be isolated using partial enzymatic digestion of the amorphous phase. The resulting type III RS consists of amylose with a relatively high degree of crystallinity.<sup>[26]</sup> In retrogradation applications, starch granules are completely broken down. As it cools, the amylose chains join via hydrogen bonds to form a stable double helix.<sup>[9]</sup> Physical modifications are applied to obtain type III RS and chemical reactions take place during the application. Foods' type III RS content can be increased by applying a high amylose content, acid and enzyme treatments, linear amylose chains, heating-cooling cycles, and oven-drying processes. Low water content, short amylose chains, and high amylopectin content, while freeze-drying processes reduce type III RS content.<sup>[23]</sup> It was found that when commercial type III RS was used in bread dough at a rate of 30%, the development time, stability, and extensibility properties were reduced. When commercial type III RS was used in bread at a rate of 30%, the RS content increased, and a higher quality product was obtained compared to type II RS-added bread.<sup>[25]</sup>

### 2.4. Type IV Resistant Starch

Among the examples of type IV RS are some of the chemically modified starch ethers, starch esters, and cross-linked starch formed by crosslinking or adding chemical derivatives.<sup>[27]</sup> Type IV RS is produced through the cross-linking of starches from rice, wheat, corn, potato, tapioca, oat, and mung beans by using sodium trimetaphosphate, sodium tripolyphosphate, epichlorohydrin, or phosphoryl chloride.<sup>[28]</sup> While forming type IV RS, chemical reactions occur in cross-linking or substitution applications. These chemical reactions occur in the hydroxyl groups of amylose and amylopectin.<sup>[29]</sup> Highly cross-linked starch loses its swelling property during cooking. As a result, highly cross-linked starch remains in the granule form with low enzymatic sensitivity after cooking and cannot be hydrolyzed by amylases.<sup>[18]</sup> The content and digestibility of type IV RS vary depending on the botanical origin of the starch, the type and amount of modifying agents, and the chemical bonds formed.<sup>[30]</sup> It was determined that when 30% commercial type IV RS was used in bread dough, water absorption and development time were similar to those of dough using white flour, but gluten strength and extensibility were reduced. When 30% commercial type IV RS was used in bread, the fiber content increased, but the specific volume and technological quality decreased.[25]

### 2.5. Type V Resistant Starch

Type V RS is formed because of the formation of amyloselipid complex resistant to enzymatic digestion. Lipids (fatty acids, monoglycerides, etc.) form complexes between the hydrocarbon moieties present in the helix space of amylose. This structure includes unbranched glucan chains. Since the amylose-lipid complex forms a complex structure, its hydrolysis by amylase is prevented, which limits the swelling of starch granules.<sup>[18,20]</sup> The resistance to the digestion of type V RS varies depending on the lipid and amylose molecular structures, the crystal structure of the amylose-lipid complex, and temperature.<sup>[31]</sup> Moreover, the chain length and unsaturation of fatty acids influence resistance to the digestion of type V RS. Increasing the carbon number of fatty acids increases the resistance of starch to digestion. As the unsaturation of fatty acids increases, the resistance of starch to digestion decreases.<sup>[9,31]</sup> Adding type V RS to bread increased the amount of RS in the bread but caused an undesirable texture. The occurrence of an undesirable situation in the texture was found to be associated with the high amount of RS, and it was revealed that type V RS was resistant to heat and remained in the bread after baking.[32]

## 3. Physiological Effects of Using Resistant Starch in Bread

Dietary fiber has such effects as shortening gastrointestinal transit time, increasing stool volume, being fermented colon microflora, lowering the level of low density lipoprotein (LDL) cholesterol, and lowering blood glucose level. Thanks to these effects provided by fiber, it can be used as a component in the development of functional foods to prevent noncommunicable diseases.<sup>[33]</sup> RS is recognized as a dietary fiber by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives and the CODEX Alimentarius Commission.<sup>[34,35]</sup> RS is added to foods as a functional ingredient to increase their fiber content.<sup>[11]</sup>

Bread consumed in many ways worldwide is an essential source of starch and energy.<sup>[36]</sup> Although bread is a primary food in the human diet, the amount of fiber in white bread is less than 2.5%. White wheat bread is widely used as a high GI reference in glycemic response.<sup>[37]</sup> According to the 2019 results of the Turkey Nutrition and Health Survey (TNHS), the amount of bread consumed daily is  $226.3 \pm 144.14$  g in men, which is  $179.8 \pm 130.39$  g in women. In adults, 39.5% of the average daily energy intake is from bread and cereals.<sup>[38]</sup> On the other hand, in European countries, the amount of white bread consumed daily is 170 g.<sup>[39]</sup> The starch included in white bread is rapidly digested; thus, it leads to a high glucose and insulin response. Therefore, white bread has a high GI (>70).<sup>[40]</sup> Given the amount of consumption and high GI of white bread, it affects gastric emptying and postprandial insulin response and thus leads to the development of obesity and insulin resistance.<sup>[41]</sup> Therefore, improving the nutritional value of bread makes a great contribution to healthy nutrition. Various studies have been carried out in the literature on bread with added RS since 1994. It is becoming more popular to add RS to bread to reduce its GI.[40]

Due to its daily consumption, bread, a potential carrier of functional components, draw more and more attention. Adding fiber to bread can reduce the risk associated with gastrointestinal disorders, obesity, diabetes, and cardiovascular disease. RS is potentially important in developing various fiber-enriched foods due to its nutritional value and technological properties.<sup>[42]</sup> RS provides less energy than digestible starch. While the energy value of digestible starch is 17 kJ  $g^{-1}$  energy, that of the RS is 8–10 kJ  $g^{-1}$ .<sup>[6]</sup> Dietary fiber and glycemic indices of bread made using type II RS at 0% (control), 10%, 20%, and 30% as a substitute for wheat flour have been investigated. The fiber contents of bread whose type II RS content is 0%, 10%, 20%, and 30% are  $6.6 \pm 0.1$ ,  $9.5 \pm 0.1$ ,  $17.0 \pm 0.7$  and  $26.6 \pm 1.8$  g, respectively. The estimated GI of bread whose type II RS content is 0%, 10%, 20%, and 30% are 100, 92.4  $\pm$  2.3, 83.5  $\pm$  2.3 and 76.5  $\pm$  0.6, respectively.<sup>[43]</sup> In a study, it was found that when high amylose corn starch was added to wheat flour, the fiber content increased from 3.4 to 6.7.<sup>[44]</sup> In another study, in which the effects of RS added to bread on total dietary fiber were investigated, type IV RS was added at the rates of 5%, 10%, 15%, 20%, and 25%. It was observed that RS content at these rates led to a linear increase of 4%, 4.3%, 6.1%, 7.3%, and 9.2% in the total dietary fiber, respectively.<sup>[45]</sup> Fifteen gram of RS added to a 45 g serving of breadsticks reduced the in vitro GI by approximately 15%. The breadsticks' nutritional composition analysis demonstrated that RS led to a reduction in energy and total sugar due to the dilution effect of the protein content.<sup>[46]</sup> After the in vitro GI of control bread and bread containing 15%, 20%, and 25% type IV RS was calculated, it was determined that the GI of bread with RS was significantly lower. The results of the study showed that the bread containing 20% or more type IV RS was in the category of medium or low GI compared to white bread. There was an increase in the dietary fiber content of the bread with RS and the staling time of these bread was longer.<sup>[47]</sup> The effects of bread containing  $\beta$ -glucan, RS, or both on the GI and glycemic load in healthy individuals were investigated. The GI and glycemic load of the bread containing RS,  $\beta$ -glucan, or both were significantly lower than those of white bread.<sup>[48]</sup> In another study of bread made from barley flour, RS added to the bread significantly contributed to total dietary fiber.<sup>[36]</sup> The GI of bread

with high amylose corn starch was investigated. In the study carried out, the GI of white bread was determined as 100, while the GI of bread with high amylose corn starch was determined as  $60 \pm 18$  (mean  $\pm$  SD).<sup>[49]</sup> Various studies reported that the GI of bread with increased amylose content showed a negative correlation with the amount of RS.<sup>[50,51]</sup> Binou et al.<sup>[52]</sup> found that the GI of bread enriched with 8.8 g RS was  $40 \pm 8$  when the glucose solution was accepted as a reference. In a study in which bread was enriched with type III RS, white bread showed a GI of 82 while RS bread presented a value of 60. According to the GI classification, white bread is in the group of foods with a high GI, while RS bread is in the group of foods with a medium GI.<sup>[53]</sup> It has also been shown that RS does not affect the GI. White bread has a GI of 100, while bread with high amylose corn starch has a GI of 99.4.<sup>[54]</sup> It was demonstrated that adding RS to bread and flour, increased the fiber content of the bread, and lowered the GI of the bread. Therefore, adding RS attracts attention as an important strategy to reduce the GI of bread. An increase in the RS content of the bread gained the bread a functional feature. Using RS in bread, an essential nutrient in the diet is expected to be effective in the treatment of common chronic diseases such as obesity and type 2 diabetes.

The addition of RS to various foods at a certain rate does not adversely affect the texture of the food. When type IV RS was added to bread at 5%, 10%, 15%, 20%, and 25%, dough strength and extensibility were not affected by the addition of RS. Bread volume was not adversely affected until it reached 20% RS. When the sensory properties of bread are examined, it has been reported that bread containing 15%, 20%, and 25% RS is not different in terms of taste, texture, or general taste.<sup>[45]</sup> It has been shown in various studies that the rheological properties of the dough and the quality of the bread are not adversely affected when the amount of RS added to the bread reaches 30%.[55,56] In another study, when the RS addition rate was 10% and 30%, the bread made was like the control group in terms of volume and appearance. However, it has been reported that when the RS ratio reaches 50%, the volume of the bread decreases, and its texture is adversely affected.<sup>[44]</sup> The use of RS in bread and bakery products at certain rates will enable the development of new functional products for the food industry and consumers.

# 4. Effects of Consumption of Resistant Starch in Bread and Other Products on Glycemic Response and Appetite

### 4.1. Glycemic Response

The same amount of carbohydrates leads to different blood glucose responses in different foods is defined as GI.<sup>[57]</sup> Foods with a low GI cause a lower glycemic response because they are digested and absorbed more slowly, while foods with a high GI cause a higher glycemic response because they are digested and absorbed quickly. Consumption of low-GI foods reduces the rate of glucose absorption. It causes a lower increase in circulating insulin and related gastrointestinal hormones, such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) called incretins.<sup>[41,57]</sup> A diet with a high GI may increase insulin secretion, which may lead to postprandial hyperinsulinemia. The continuation of hyperinsulinemia gets into a vicious circle with peripheral cell insulin receptor downregulation and thus triggers insulin resistance.<sup>[57]</sup> Glycemic load (GL) is considered a concept that summarizes both GI and carbohydrate content, representing a food's overall glycemic effects. Increasing the GL of the diet results in predictable increases in glycemia and insulinemia. Therefore, foods' GI and GL values should be evaluated together.<sup>[58]</sup> To calculate the GL, multiply the GI by one gram of carbohydrate per serving of the test item. One GL unit describes the glycemic impact of 1 g of carbohydrate from a reference food, such as white bread.<sup>[59]</sup> Foods having a high GI typically have a high GL. In the evaluation of GL, 0-10 is classified as low, 10-20 as medium, and 20 and above as high.<sup>[58]</sup> It was determined by Hefni et al. that the GL of breads with increased RS content from various cereals was low and the GI was in the medium category.<sup>[60]</sup> Similar results were obtained in another study that was conducted. Breads with a higher RS content had a lower GL.<sup>[61]</sup> As a result of the enrichment of rice flour with RS, GL and GI values decreased in parallel with the increase in RS.<sup>[62]</sup> Dietary fiber, starch type, gelatinization, and the process of making starches water-soluble are the modifying factors of the GI and GL.<sup>[63]</sup> Therefore, increasing the fiber content of foods is important, and RS is added as a functional component to increase the fiber content.<sup>[11]</sup>

RS reduces not only the GI of foods but also the glycemic response of individuals after consumption. It has been shown that the consumption of foods containing RS increases glucose and insulin levels less than the consumption of foods containing rapidly digestible starch.<sup>[64]</sup> RS which can be fermented in the colon positively affects the growth of species such as Bifidobacterium and Lactobacillus, and because of fermentation, it turns into short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. The increase in colonic fermentation of RS increases the production of SCFAs, thus affecting increases glucose uptake in the muscles and liver, releasing intestinal hormones, and increasing insulin sensitivity. After the consumption of RS, there is a decrease in appetite parameters due to the decrease in food intake caused by changes in glucose and insulin levels.<sup>[65]</sup> The RS effect on the glycemic response occurs through metabolic pathways in the liver and pancreas. RS affects glucose homeostasis through upregulation of the expression of genes involved in glycogen synthesis (GS2 and GYG1) and insulin-inducing genes (Insig-1 and Insig-2) in the liver. In addition, it reduces gluconeogenesis by downregulating glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) enzymes involved in glycogen synthesis. RS also shows its effect on the glycemic response in metabolic pathways in the pancreas. It improves insulin transport by upregulating the expression of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) in the pancreas. RS improves glucose response by upregulating pancreatic duodenal homeobox-1 (PDX1), glucose kinase, and glucose transport 2 (GLUT2) expression. Figure 1 shows the metabolic pathways in which RS is effective in the glycemic response.<sup>[66]</sup>

Intake of 6–12 g of type II RS in a meal has been shown to have positive effects on postprandial glucose and insulin levels.<sup>[67]</sup> EFSA approved the following health declaration: "Replacing digestible starch with RS lowers postprandial blood glucose levels." However, this only occurs when the total RS content in foods is at least 14% of the total starch.<sup>[68]</sup> The effects of RS on the glycemic

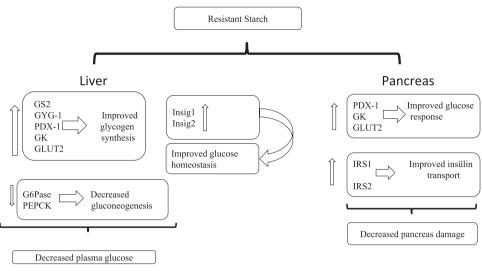


Figure 1. Metabolic pathways of the effect of resistant starch on glycemic response.

response have been investigated in various studies. While acute consumption of bread containing type III RS did not affect glucose response, it was revealed that insulin response was lower than white bread.<sup>[69]</sup> It was observed that bread containing 60% high amylose corn starch significantly reduced both glucose and insulin response compared to white bread.<sup>[32]</sup> In a study in which individuals with type 2 diabetes consumed a bagel containing 20% RS, glucose and insulin responses were found to be lower than the control bagel.<sup>[70]</sup> When the acute consumption of highamylose wheat starch bread by healthy individuals is examined, glucose and insulin responses are lower than bread containing low-amylose wheat starch.<sup>[71]</sup> Postprandial glucose and insulin levels were investigated after the consumption of meals containing type II RS in healthy individuals, which showed that their postprandial glucose and insulin levels were significantly lower than those of the individuals in the control group.<sup>[72]</sup> In a study conducted with overweight individuals at risk for type 2 diabetes, it was indicated that while consumption of bagels containing RS improved fasting insulin sensitivity in individuals, it increased glycemic efficiency by reducing the amount of insulin required to manage postprandial blood glucose level.<sup>[73]</sup> In various studies enriching bread with type II RS, it was found that RS did not affect glucose and insulin response.<sup>[74-76]</sup> Table 4 presents the summary of studies examining the glycemic response of RS bread and other products. In a meta-analysis in which the effects of RS supplementation on glucose, insulin, and insulin resistance in overweight and obese individuals were investigated, the results showed that RS supplementation could improve fasting glucose, fasting insulin, insulin resistance, and sensitivity, especially in overweight or obese individuals with diabetes.<sup>[77]</sup> After the effects of RS on glycemic response were investigated, it was observed that RS supplementation had a positive effect on postprandial glucose and insulin levels. Foods consumed in these studies often include type II RS. However, there are some other studies, in which foods including type III RS are consumed. In studies in which the effect of RS on glycemic response in healthy, prediabetic or obese individuals was investigated, the effects of RS supplementation on glycemic response were also investigated. In addition, the sample size of these studies was quite low. Therefore, the effects of RS on glycemic response should be demonstrated through randomized, controlled clinical studies with larger sample sizes and longer intervention duration. Further studies should be conducted to investigate the effects of type II RS and the effects of type III RS and type IV RS on glycemic response.

### 4.2. Appetite

Because RS is not absorbed in the small intestine and is fermented in the large intestine, it turns into SCFA. SCFA activates G protein-dependent receptors (GPR41 and GPR43) on intestinal epithelial cells. Activation of G protein-dependent receptors with consuming RS increases peptide YY (PYY), GLP-1, and leptin levels. As a result, appetite is suppressed, food intake decreases, and satiety increases.<sup>[78]</sup> The effects of RS consumption on appetite are shown in Figure 2.<sup>[64]</sup> Studies investigating the effects of RS on appetite have been compiled in the literature. In a study in which glucose solution was used as a control parameter, consumption of bread containing RS significantly decreased cravings to eat and increased satiety after the 15th minute, which lasted up to the 180th minute, compared to consumption of glucose.<sup>[52]</sup> After consuming the test meal with a type 2 RS bagel, GLP-1 and GIP levels were found to be lower in the first 3 h compared to the meal containing a control bagel. However, with the effect of the second meal, GLP-1 and GIP levels increased after the 3rd hour compared to the test meal with the control bagel. The data obtained in the study results are not statistically significant.<sup>[70]</sup> Bread with high amylose wheat starch consumed by healthy individuals showed a decrease in GIP and GLP-1 levels compared to bread with low amylose wheat starch. However, bread with RS did not affect ghrelin levels and subjective appetite measurements.<sup>[71]</sup> There are also various studies in the literature showing that there is no significant difference in appetite parameters and subjective measurements in those who consume bread containing RS compared to those who consume

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Product containing resistant starch	Types of resistant starch	Subject	Dose/intervention period	Inferences	References
Bread	High-amylose maize starch	Healthy volunteers (6 men and 2 women; mean age: 25 years, mean body weight 55±70 kg)	White bread (WB): 60 g High amylose wheat bread (HAWB) (%40 resistant starch): 60 g	Plasma glucose was significantly lower with HAWB than with WB at 90 and 105 min. Mean insulin responses to HAWB were significantly lower than for WB within the 90–200 min period.	[49]
Bread	High-amylose corn starch	Healthy volunteers (13 men and 12 women; mean age: 41.1 years, mean BMI: 27.5 kg m <sup>-2</sup> )	Glucose (1 g glucose/kg body weight) Five breads (1 g carbohydrate/kg body weight) made with 70% amylose corn starch, standard corn starch (30% amylose), and blends of the two starches (40%, 50%, and 60% amylose starch)	The lowest area under the curve (AUC) occurred after the 60% and 70% amylose starch bread. Insulin response and AUC were significantly lower after the 60% and 70% amylose starch bread than after the glucose or the other bread.	[50]
Bread	High-amylose resistant starch	Healthy subjects (7 men and 7 wornen, age range of 20—35 years, mean BMI: 22.2 kg m <sup>-2</sup> )	White bread (WB): 50 g available starch Whole grain wheat with elevated arrylose content (EAW): 50 g available starch (%13 resistant starch)	EAW bread induced lower postprandial glucose response than WB during the first 120 min, but there were no significant differences in insulin responses.	[13]
Bread	High-amylose waxy maize starch	Normoglycemic subjects (5 men and 5 women, mean age: 27 years, mean BMI: 24.5 kg m <sup>-2</sup> )	Clucose: 50 g β-glucans bread: 50 g of available carbohydrates (6 g β-glucans) RS bread: 50 g of available carbohydrates (8.8 g RS)	Ingestion of $\beta$ -glucans and RS bread elicited a lower glycemic response compared to glucose. There were no significant differences in insulin response between the two breads.	[52]
Bread	Type III	Nondiabetic subjects (11 men and 26 women, age range of 22—59 years, BMI range of 24–29.99 kg m <sup>-2</sup> )	White bread: 50 g of available carbohydrates (88 g/0.7 g RS) RS bread: 50 g of available carbohydrates (102 g/2.5 g RS)	RS bread presented a significantly lower postprandial glucose response at 60, 90, and 120 min. No differences were observed in insulin response.	[53]
Bread	Type III	Untreated borderline diabetes mellitus subjects (9 men and 11 women; mean age: 50.5 years)	White bread: 140 g RS bread: 140 g (6 g resistant starch)	There is no significant difference in AUC glucose between RS bread and white bread. After consuming RS bread, the AUC insulin is significantly lower than on white bread.	[69]
Bread	Type V (complexing high-amylose maize starch with palmitic acid)	Healthy subjects (20 men, age range of 19—38 years, BMI range of 21.0—42.8 kg m <sup>-2</sup> )	White bread: containing 50 g of starch RS bread: containing 50 g of starch (%60 resistant starch)	The postprandial plasma-glucose and insulin responses after ingesting bread made from RS were reduced to 55% and 43%, respectively, when compared with those after ingesting white bread.	[32]
Bagel	Type II	Type II diabetes subjects (5 men and 7 women, age range of 50–66 years, mean BMI: 33.1 kg m <sup>-2</sup> )	Control bagel: 50 g of available CHO RS bagel: 50 g of available CHO (%20 resistant starch)	The test meal with the RS bagel elicited a lower glucose incremental area under the curve (iAUC) and insulin iAUC than the test meal with the control bagel.	[70]

Table 4. The effect of bread and other products with resistant starch on glycemic response.

\_\_\_\_\_Starch

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Product containing resistant starch	Types of resistant starch	Subject	Dose/intervention period	Inferences	References
Bread	High-amylose wheat starch	Healthy nondiabetic subjects (5 men and 15 women, mean age: 30 years, mean BMI: 23 kg m <sup>-2</sup> )	High-amylose wheat refined (HAW-R) bread: 121 g (4.7 g RS) High-amylose wheat whole meal (HAW-W) bread: 121 g (3.2 g RS) Low-amylose wheat refined (LAW-R) bread: 121 g (0.4 g RS) Low-amylose wheat whole meal (LAW-W) bread: 121 g (0.3 g RS)	HAW bread had a glycemic response that was 39% less than LAW bread. Insulinemic responses were 24–30% less for HAW bread than for LAW bread. Processing of the flour (whole meal or refined) did not affect the glycemic or insulinemic response.	[1,2]
Roll	Type II	Healthy subjects (12 men and 18 women, mean age: 53.9 years, mean BMI: 26.5 kg m <sup>-2</sup> )	Control roll: 2–3 g RS per day/7 days RS roll: 14–19 g RS per day/7 days Women were asked to eat three rolls per day (a half roll at breakfast and lunch, two at dinner) while men were asked to eat four rolls per day (one at breakfast and lunch, two at dinner).	Consumption of RS rolls for one week resulted in reduced postprandial glucose and insulin responses relative to conventional wheat when participants were provided with a standard breakfast meal containing the respective treatment rolls (RS-enriched or conventional wheat).	[72]
Bagel	Type II	Diabetic subjects (16 men and 8 women, mean age 55.3 years, mean BMI 30.2 kg m <sup>-2</sup> )	Control bagel: 124.2 g (6.83 g RS)/56 days RS bagel: 119.8 g (25.4 g RS)/56 days	The RS bagel treatment resulted in significantly lower fasting, and 3-h insulin incremental areas under the curve and fasting insulin resistance than did the control bagel treatment. Fasting and postprandial OGTT glucose concentrations did not differ between the RS and control bagel treatments on study days.	[73]
Bread	Type II	Overweight (8 men and 9 women, mean age: 36.6 years, mean BMI: 37.7 kg m <sup>-2</sup> )	Control Bread: 3 slices per day (2.64 g RS)/6 weeks RS bread: 3 slices per day (12.39 g RS)/6 weeks	There were no significant differences in the subjects' fasting plasma glucose and insulin levels due to the consumption of the RS bread vs the control bread.	[74]
Bread	Type II	Healthy subjects (9 men and 10 women, mean age: 23 years, mean BMI: 22.2 kg m $^{-2}$ )	White bread: 50 g available starch (1.2 RS) RS bread: 50 g available starch (15 RS)	There were no significant differences in the subjects' glucose and insulin response due to the consumption of the RS bread vs the control bread.	[75]
Bread	Type II	Healthy subjects (7 men and 5 women, mean age: 24 years, mean BMI: 23.3 kg m <sup>-2</sup> )	White bread: 95.6 g (1.1 g RS) RS bread: 114.6 g (6.0 g RS)	There was no significant difference between white bread and resistant starch bread in terms of postprandial glucose and insulin response.	[76]

Table 4. (Continued).

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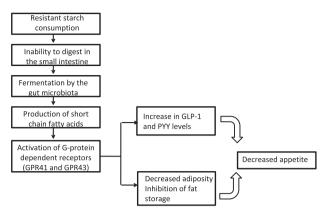


Figure 2. Effect of resistant starch consumption on appetite.

white bread.<sup>[75,76]</sup> In a double-blind, randomized controlled study, participants consumed rolls made with type II RS-enriched wheat flour or wheat flour alone for 7 days. A significant increase in PYY levels and a significant decrease in GIP levels were observed in the group consuming type II RS. However, perceptions of hunger or satiety reported using visual analogue scale (VAS) did not differ between groups.<sup>[79]</sup> Table 5 presents the summary of studies examining the appetite response of RS bread and other products. A meta-analysis in which the effects of acute RS consumption were investigated showed that consumption of 25 g or more of RS resulted in a significant reduction in appetite. It also revealed that type II RS was more effective than other RS types.<sup>[80]</sup> Although several studies have been conducted to investigate the effects of using RS in different products on appetite, the number of studies in which the effects of consuming RS as bread on appetite is investigated is very few. When the studies in which the effects of

Table 5. The effect of bread and other products with resistant starch on appetite.

Product with resistant starch	Types of resistant starch	Subject	Dose/intervention period	Inferences	Reference
Bread	High-amylose waxy maize starch	Normoglycemic subjects (5 men and 5 women, mean age: 27 years, mean BMI: 24.5 kg m <sup>-2</sup> )	Glucose: 50 g β-glucans bread: 50 g of available carbohydrates (6 g β-glucans) RS bread: 50 g of available carbohydrates (8.8 g RS)	There were no significant differences in ghrelin, GLP-1 or PYY between the two breads. A significantly lower desire to eat and higher fullness was detected 15 min after bread consumption and until 180 min compared to glucose solution.	[52]
Bagel	Туре II	Type II diabetes subjects (5 men and 7 women, age range of 50–66 years, mean BMI: 33.1 kg m <sup>-2</sup> )	Control bagel: 50 g of available RS bagel: 50 g of available CHO (%20 resistant starch)	While the GLP-1 and GIP levels were lower at 0–180 min after the test meal with the RS bagel, compared to the meal with the control bagel; at 180–300 min, the GLP-1 and GIP levels of the test meal with the RS were higher than the meal with the control bagel. However, the results are not statistically significant.	[70]
Bread	High-amylose wheat starch	Healthy nondiabetic subjects (5 men and 15 women, mean age: 30 years, mean BMI: 23 kg m <sup>-2</sup> )	High-amylose wheat refined (HAW-R) bread: 121 g (4.7 g RS) High-amylose wheat whole meal (HAW-W) bread: 121 g (3.2 g RS) Low-amylose wheat refined (LAW-R) bread: 121 g (0.4 g RS) Low-amylose wheat whole meal (LAW-W) bread: 121 g (0.3 g RS)	The consumption of HAW breads resulted in a 30% lower GIP and GLP-1 iAUC compared with the LAW breads. However, the HAW breads did not influence plasma ghrelin or subjective measures of satiety or cravings during the postprandial period.	[71]
Bread	Туре II	Healthy subjects (9 men and 10 women, mean age: 23 years, mean BMI: 22.2 kg m <sup>-2</sup> )	White bread: 50 g available starch (1.2 RS) RS bread: 50 g available starch (15 RS)	There were no significant differences in the subjects' GLP-1 and GLP-2 due to the consumption of the RS bread versus the control bread.	[75]
Bread	Туре II	Healthy subjects (7 men and 5 women, mean age: 24 years, mean BMI: 23.3 kg m <sup>-2</sup> )	White bread: 95.6 g (1.1 g RS) RS bread: 114.6 g (6.0 g RS)	Compared to those consuming white bread, in those consuming bread containing RS their feeling of fullness (satiety), and hunger are not significant.	[76]
Roll	Туре II	Healthy subjects (12 men and 18 women, mean age: 53.9 years, mean BMI: 26.5 kg m <sup>-2</sup> )	Control roll: 2–3 g RS per day/7 days RS roll: 14–19 g RS per day/7 days Women were asked to eat three rolls per day (a half roll at breakfast and lunch, two at dinner) while men were asked to eat four rolls per day (one at breakfast and lunch, two at dinner).	Results indicated that the peak level of peptide YY increased, while the peak level and iAUC of GIP decreased after ingesting the RS roll. PYY iAUC showed no significant difference between treatments. There were no significant differences between RS and control for ghrelin, leptin, and GLP-1.	[79]

BMI, body mass index; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; PYY, peptide YY; RS, resistant starch.

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RS on appetite were investigated, it was observed that consuming RS caused a decrease in appetite and an increase in satiety by affecting both subjective appetite parameters and gastrointestinal hormone levels. However, there are studies in the literature indicating that RS did not affect appetite and gastrointestinal hormone levels. It is thought that the type and dose of RS consumed, sample size and intervention duration affected the emergence of these results. More research is needed to reveal the effects of RS on appetite metabolism. Therefore, we recommend that randomized, controlled clinical studies with larger sample sizes and longer intervention duration should be conducted.

### 5. Conclusion

In recent years, with the positive effects of RS on health, there has been a significant increase in the number of studies in which the effects of RS supplementation are investigated. In this review study, it was revealed that RS had positive effects on blood glucose, insulin, and gastrointestinal hormone levels. Although some studies have shown that RS has no effect on the GI or glycemic response, many studies have shown that RS improves the glycemic response. With the addition of RS to the bread, the GI of the bread decreases. In this context, RS is a candidate to become a popular nutritional component and may play a significant role in developing new functional foods due to both its physiological and functional properties. Given the physiological properties of RS and the quantitative importance of bread in the diet, bread is considered the major source of RS. Adding RS to bread, a food whose consumption rate is high is thought to be a potential component in the treatment of chronic diseases such as obesity, insulin resistance, and diabetes. The use of RS in bread and bakery products is important for developing new functional products for the food industry and consumers. Further detailed research on the most beneficial RS type and dosage, particularly on glycemic response and appetite, should be done to provide more clarity on the matter.

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### Conflict of Interest

All authors declare that they have no conflict of interest.

### **Author Contributions**

T.T.: Conceptualization, methodology, investigation, writing - original draft preparation. M.F.: Writing-review and editing.

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appetite, bread, glycemic index, prebiotic, resistant starch

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