In the last century, starch present in foods was considered to be completely digested. However, during the eighties, studies on starch digestion started to show that besides digestible starch, which could be rapidly or slowly hydrolysed, there was a variable fraction that resisted hydrolysis by digestive enzymes. That fraction was named resistant starch.
(RS) and it encompasses those forms of starch that are not accessible to human digestive enzymes but can be fermented by the colonic microbiota, producing short chain fatty acids. RS has been classified into five types, depending on the mechanism governing its resistance to enzymatic hydrolysis. Early research on RS was focused on the methods to determine its content in foods and its physiological effects, including fermentability in the large intestine. Later on, due to the interest of the food industry, methods to increase the RS content of isolated starches were developed. Nowadays, the influence of RS on the gut microbiota is a relevant research topic due to its potential health-related benefits. This review summarizes over 30 years of investigation on starch digestibility, its relationship with human health, the methods to produce RS and its impact on the microbiome.

Keywords: Digestibility; microbiome; resistant starch; slowly digestible starch; satiety

Starch overview

Starch hydrolysis

  Overview

  Resistant starch (RS)

  Slowly digestible starch (SDS)

Modulating starch hydrolysis
Starch overview

Starch is among the three main polysaccharides present in nature, but in contrast with cellulose and chitin that are structural molecules, starch is a storage carbohydrate. From a functional point of view, starch present in cereals (maize, wheat, rice, etc.), legumes (beans, lentils, chickpea, etc.), tubers and roots (potato, sweet potato, cassava, etc.) and unripe fruits (plantain, mango, apple, etc.) gives functionality (viscosity, water retention, mouthfeel, etc) when they are cooked and consumed directly or used to prepare processed foods as bakery products, tortillas, pasta, etc.\textsuperscript{1,2} In both forms of consumption, the nutritional aspect of starch is most important, where the product type (food matrix), processing (e.g. mixing, cooking type) and other ingredients in the product (lipids, proteins, polyphenols) have marked influence on starch digestibility and the glycaemic response.\textsuperscript{3} Also, starch can be isolated and used as an ingredient in foods, but under this application type it is mainly used to impart functionality, without paying much attention to the nutritional aspect. However, in the last decade, there has been interest in the development of starch ingredients with reduced susceptibility to hydrolysis by digestive
enzymes, resulting in slow and limited glucose supply to the circulation. Those foods with starch of low-digestibility are attractive for people with diabetes and other obesity-related health problems.

The nutritional value of starch depends on extrinsic aspects, as mentioned above, and intrinsic features such as granule size, amylose/amylopectin ratio, chain-length distribution of amylopectin and arrangement of starch components in the granule, which are important both when foods are ingested as a complex matrix and in the production of starch ingredients with reduced glycaemic impact.

Starch is a homopolysaccharide of glucose. The polymer consists of two main components: amylose and amylopectin. Amylose is considered an essentially linear molecule where the glucose units are linked by α-(1-4) bonds; however, amylose presents scarce branching points but its functionality corresponds to that of a linear polymer. Amylose is found in the amorphous lamella of the starch granule. Amylopectin is the branched component with linear sections where glucose units are linked by α-(1-4) bonds, and branching points consist of α-(1-6) bonds. Amylopectin is responsible for the crystalline lamella of the granule, and its branching points are part of the amorphous lamella. The presence of amorphous and crystalline lamellae in the starch granule confers this biopolymer a semi-crystalline entity. The semi-crystalline characteristic of starch granule, i.e. the arrangement of starch components, is an important determinant of its digestibility, more than the crystallinity level. Recently, it was reported that flexible α-glucans obtained after gelatinization of waxy rice starch are exposed in the periphery of the granules and are the primary substrate of α-amylase; those flexible α-glucans are rapidly cleaved in the initial stage of the hydrolysis. However, considering the intrinsic
heterogeneity of individual starch granules and the even larger differences observed among granules of different botanical sources, the mechanisms of the hydrolysis by \( \alpha \)-amylase deserve more detailed investigation, given the important physiological implications of starch digestion.\(^7\)

Starches are classified according to the amylose/amylopectin ratio: those starches with 25-30 % amylose and 70-75 % amylopectin are usually named as “normal” starches; some starches present high amylopectin levels (98-99 %) and are called “waxy” starches; a third group includes starches with high amylose contents (50-70 %), e.g. Hylon® maize starches.

Starch isolated from different botanical sources can present minor constituents, as proteins, lipids and phosphate groups. Potato starch present phosphate monoesters and phospholipids that give special functional characteristics to gels made with this starch. Potato starch gels show high peak viscosity and high transparency attributed to the phosphate groups present at the branching points of amylopectin. The lipids present in some starches, such as maize and rice, can form inclusion complexes with amylose and modify the digestibility of the polysaccharide, since the network produced during gelation (food matrix) can restrict the accessibility of the polymer to the enzymes responsible for the hydrolysis process. Also, the interactions between phosphate groups and amylopectin chains change the starch structure in such a way that it is not efficiently recognized by amylolytic enzymes, thus becoming a bad substrate.

**Starch hydrolysis**

**Overview**
The hydrolysis of starch present in the foods has been a hot topic for more than twenty-five years (Figure 1). Before, it was accepted that all starch present in foods was hydrolysed, first by the action of salivary $\alpha$-amylase and later at small intestinal level by pancreatic $\alpha$-amylase, producing, maltose, maltotriose and other branched oligosaccharides called $\alpha$-limit dextrins, which are further converted into glucose by the action of the brush border enzymes maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI).\textsuperscript{8} Initially, following an intense period of analytical controversy, a European Concerted Action (EURESTA) was undertaken\textsuperscript{5}, confirming that a significant fraction of starch in foods is not digested and absorbed in the small intestine, reaching the colon where it is fermented to variable extent by the microbiome. This fraction was called resistant starch (RS).\textsuperscript{9} From that moment, many research groups were interested in the classification of RS types\textsuperscript{9}, methods to determine RS\textsuperscript{10-14} and methods to produce resistant starch-rich ingredients\textsuperscript{15-19} using starch from different botanical sources and treatments. Starch digestibility is associated with the proportion of starch that is absorbed as in the small intestine. The kinetic component of starch digestion is mainly associated with so-called glycaemic index (GI), which has strong influence on the postprandial metabolism. The GI is used to quantify the postprandial blood glucose response to starchy foods and it is a tool for their characterization and classification in terms of the physiological response they elicit.\textsuperscript{20,21}

**Resistant starch (RS)**

From the publication of a concerted definition of RS as “the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals”\textsuperscript{9} there has been sustained interest on this fraction. Recently, RS was included as part of the dietary fibre definition and it has been associated with weight management benefits, among
other potential effects. Diverse reviews on RS have been published over the years. Most recent publications classify RS in five different categories:\textsuperscript{22-25}

RS1: physically inaccessible starch, i.e. entrapped in a mechanically resistant cellular matrix.

RS2: native starch granules (raw) with particularly organized structure.

RS3: retrograded starch, produced after cooking and cooling of starchy foods

RS4: chemically modified starch, with the formation of “new” chemical bonds.

RS5: amylose-lipid complexes and resistant maltodextrins.

Englyst et al.\textsuperscript{11} classified starch digestion according to the rate and extent of its hydrolysis after ingestion. Following their in vitro methodology for the evaluation of starch digestibility, the fraction that resists a 120-min hydrolysis treatment was classified as RS. Such a cut-off was introduced by comparison with in vivo digestibility estimations in ileostomy patients. Controversies emerged when RS was included in the dietary fibre concept since it is suggested that the food should be analyzed “as eaten” i.e. without additional heating that disorganizes the starch structure (gelatinization), as indicated in the official method. The basis for this proposed modification is that upon heating granular RS fractions become available starch and is therefore not determined as part of dietary fibre. Also, 4 h instead of 16 h of hydrolysis by the enzyme cocktail was proposed to better mimic physiological conditions during digestion\textsuperscript{26} but, evidently, further studies of these aspects are necessary.

In our opinion current trends in RS investigation include the use of alternative starch sources (e.g. banana) and methods to produce RS-rich powders that can be used as ingredient in foods with high indigestible carbohydrates content; the effect of the food matrix and starch interactions with diverse macromolecules and polyphenols that restrict or
inhibit starch digestion and absorption of the released glucose; the relationship between RS structure and digestibility with application in the development of tailor-made starches; the relationship between the intake of RS and gut microbiota composition and metabolism and its connection with the host health. These topics will be addressed in the following lines.

**Slowly digestible starch (SDS)**

The Englyst’s classification of starch fractions based on the hydrolysis rate and extent also considered a fraction that is slowly digested thus resulting in sustained glucose release in the small intestine. The importance of this fraction, named SDS (slowly digestible starch), resides in that its ingestion results in no sharp postprandial blood glucose peak with a concomitant low insulinaemic response; the control of glycaemic and insulinaemic postprandial responses is an issue of relevance for both diabetics and healthy people. The slow digestion feature of this starch fraction of foods is related to many factors, such as structural changes produced during processing, cooking and storage. Zhang and Hamaker\(^\text{27}\) proposed two fundamental mechanisms involved in the slow digestion of starch: a) modification of starch structure that decreases enzyme susceptibility; b) change in the starch structure that limits the rate of enzyme hydrolysis. In addition, certain structural characteristics of the food matrix are also relevant, as those represented by a limited physical accessibility of starch due to its entrapment within rigid cell walls in legumes,\(^\text{28}\) a phenomenon that has significant analytical and physiological consequences.\(^\text{29-31}\) Zhang and Hamaker\(^\text{27}\) have stated that there is no in vivo method capable of evidencing the real SDS content of foods. By definition, SDS is slowly digested resulting in sustained glucose liberation throughout the whole length of the small intestine. SDS is thus considered a nutritional concept that is quantified as a physiological response. These characteristics
hamper a relevant in vitro quantification of SDS, keeping in mind the complexity of the human body response to dietary carbohydrates. As a matter of fact, the current in vitro quantification of the various fractions comprising the digestible starch content of foods has been questioned recently, proposing that the nutritional classification of starches should be modified with attention to parameters derived from advanced kinetic analyses of starch digestion, instead of relaying on in vitro RDS, SDS and RS measurements only. As consumption of low glycemic index foods, i.e. those rich in SDS, begun to be increasingly associated with improved intestinal and systemic health, more investigation was necessary to improve the quantification of this fraction. In this sense, Zhang and Hamaker proposed the extended glycaemic index (EGI) concept to determine in vivo the extent of glucose release over a prolonged period of time in different foods and SDS-rich ingredients. Also, the authors proposed the EGI to evaluate the nutritional characteristics of foods on the basis of their glycaemic carbohydrate content.

**Modulating starch hydrolysis**

**Foods (processing and food matrix)**

The unit operation for food processing (traditional and novel) can be manipulated to control the starch digestion present in different foods, such as bakery products, pasta, snacks, parboiled rice, potato, breakfast cereals, cooked beans, etc. Also, storage conditions can modify starch digestibility. Food processing involves gelatinization of the starch present which later undergoes a re-ordering of its polysaccharide components, a process that starts immediately upon cooling. During this re-ordering, generally named “retrogradation”, amylose chains lixiviated from the starch granule in the gelatinization process, are organized in a parallel or elongated arrangement, a phenomenon that is potentiated by
storage. This arrangement produces a structure that is refractory to the action of digestive enzymes and thus contributes to the RS fraction. Also, native (ungelatinized) starch granules can contribute to the overall RS fraction present in a food. The thermal processing of starchy foods in the presence of lipids can produce amylose-lipid complexes that also form part of the RS fraction. The presence of diverse components in the food matrix, such as proteins and lipids that can interact with starch, modify the digestibility of the polysaccharide and the sensory characteristics of the products. For example, bread baked from just wheat flour, salt, water and yeast, becomes retrograded upon storage, a process that is easily noticed by the increased hard texture of the bread; however, on the other hand, bread baked using lipid ingredients undergoes slow retrogradation, as consequence of the more limited interactions occurring between gelatinized starch chains. Nonetheless, the use of lipids leads to the formation of amylose-lipids complexes, with reduced starch digestibility. Partially gelatinized starch is frequently present in foods. It retains the crystalline structure (mainly cereal starches with A-type) and contributes to SDS fraction. The presence of non-starch polysaccharides (e.g. cellulose, hemicellulose, gums), proteins and lipids in the food matrix can decrease the access of the digestive enzymes to gelatinized starch and results in slow digestion. Cooked legumes, such as common beans, contain partially gelatinized starch granules entrapped by mechanically resistant cell walls which, as mentioned earlier, restrict amylolysis with concomitant slow digestion. The border between RS and SDS fractions is not completely defined and depends on diverse extrinsic factors as chewing time, foods included in the meal, characteristics of the digestive system (diseases, drug treatments), etc. The physical structure of the food matrix is another characteristic that may contribute to a slow digestion of starch and may be controlled.
through the cooking degree. Pasta, for instance, exhibits a dense and compact structure that results in a remarkable slow starch digestion rate and glycaemic response.\cite{32,33}

**Ingredients (methods to produce RS and SDS)**

The first strategy to produce a RS-rich powdered ingredient included the use of high-amylose maize starch and repeated heating-cooling cycles; this process involves structural disaggregation of linear chains that are reorganized in a new structure that is resistant to hydrolysis by digestive enzymes. In the same sense, RS production has been achieved through the generation of $\alpha$-glucan linear chains by means of debranching enzymes, as isoamylase and pullulanase that hydrolyze $\alpha$-1-6 bonds in amylopectin, and can be later re-arranged in a retrograded structure. The introduction of chemical groups in the starch structure also increases the RS fraction. Chemical modification of starch as crosslinking, acetylation, oxidation, hydroxypropylation, esterification and combinations of them produce RS;\cite{34-37} however, the level of reagents used in these modification processes is regulated and therefore the chemical approach is not easy to implement. Physical modification of starch, as for instance by hydrothermal treatments where annealing is included, has been used to produce RS. Annealing implies an excess of water (> 65% w/w) and temperatures below the gelatinization point but above the glass transition temperature\cite{38}, conditions used to destroy the granular structure resulting in rearrangement of the starch structure that is responsible for enhanced resistance to enzymatic hydrolysis. Heating-cooling cycles are used to increase the RS content in high-amylose starch.\cite{16,39} The repeated heating of a starch dispersion produced disaggregation and breaking of both starch components (amylose and amylopectin) in shorter chains. After, the cooling down of the
shorter starch chains leads to the formation of double helices aggregates, favoring the formation of denser but smaller crystals, increasing in this way the RS content. The retrogradation (reorganization) of starch chains leads to the formation of RS fractions. The process involves three steps, namely, nucleation by intra-molecular initiation of ordered chain helical segments, propagation by the growth of crystals from the nuclei by the association of chain segments, and maturation by crystal perfection.

RS-rich powders can also be produced by inducing the formation of amylose-lipid complexes. High-amylose starch was heated, cooled and incubated with isoamylase to produce linear chains and complexed with a fatty acid (palmitic acid). The RS content in the powder, assessed as dietary fibre, was 53% and was attributed to the presence of both retrograded starch and amylose-lipid complexes; the RS-rich powder was included in a bread that induced lower postprandial plasma-glucose and insulin responses compared with a control bread. The use of conventional starches to produce RS-rich powders involves a combination of methods. Chemical (acid treatment), cooking, extrusion and hydrothermal treatment were required to produce a RS-rich powder from “normal” maize starch, as its structural features (mainly amylose/amylpectin ratio) do not allow for producing enzymatic hydrolysis-resistant entities in a single step. A recently review discusses RS preparation using different methods and factors that influence RS formation. These methods have been applied to starch isolated from diverse botanical sources; the application of any method, or combinations of them, aiming to increase the RS content depends mainly on the specific use planned for the starch powder (e.g. cooking ingredient or ready-to-eat product).

There is interest to modify the starch structure present in starchy flours, avoiding the isolation step for the production of a low-cost ingredient. Unripe plantain flours were
produced after cooking of the fruit for 5, 15 and 25 minutes in boiling water; the flours showed a decrease in RS content (around 25%). For this reason, two treatments of the flours were tested to increase the RS content. Plantain flours were modified with thermal treatments using 30% moisture content at 120 °C for 24 h; the effect of cold storage (−20 °C, 7 days) was also investigated. The raw flour analyzed according to Englyst et al.11 protocol exhibited a high RS content (65.6%), which did not change with the thermal treatment (63.6%) and the additional storage (65.1%). The second treatment (annealing) of the flours showed similar RS content, suggesting that the remaining starch structure was similar after both thermal treatments. Although a decrease in RS content was observed, these unripe plantain flours may be used directly in food applications that do not require additional cooking, e.g. smoothies, salad dressings, breakfast cereals, etc, to increase the indigestible carbohydrate content.44,45 Thermal treatments (annealing and high-moisture treatment) were applied to brown rice flour and no significant change in the molecular structure was found. This pattern can be related to the matrix in the rice flour (proteins and non-starch polysaccharides) that protect the starch granules during thermal treatment. After the treatments, starch was isolated and analyzed to evaluate the changes in its structure. The molecular weight of the polysaccharide in the treated samples showed a slight decrease without change in the average chain length; a slight disorganization in the double helices of amylopectin chains was evidenced by a decrease in the relative crystallinity and enthalpy value, assessed by differential scanning calorimetry. The granular -uncooked- starch before the Englyst’s test showed RS content between 11 and 16%, which can be considered low; however, this RS level was retained after gelatinization of the preparation, suggesting that the flours can be used in the formulation of foods that require cooking.46
Multiple research groups around the world have been working on physical and enzymatic treatments to increase the SDS fraction. Starch structure modification with amylosucrase enzyme involves elongation of the non-reducing ends of (1—4)-α-glucans with sucrose as substrate; the change in the starch structure causes amylolytic enzymes to hydrolyze the starch substrate slowly—or not at all—since both SDS and RS fractions are produced.\textsuperscript{47-50} Hydrothermal treatments, specifically heat-moisture treatment, are used to modify the starch structure and produce SDS. Native potato starch was adjusted to different water contents (20, 50 or 90\%) and oven-heated at 40, 55 or 100 °C for 12 h. It was found that the hydrothermally treated samples showed crystalline arrangement, which changes from C\textsubscript{b}-type to A-type and a short-range order was observed by differential scanning calorimetry (DSC) due to the phase transition in excess water. Two basic crystalline arrangement types are found in natural starches: A-type is due to arrangement of short chains and B-type to long chains; the C-type is a mixture of A- and B-types and it is subdivided in Ca-type or Cb-type, depending on the predominant crystallinity feature (A- or B-type, respectively). The highest SDS yield was recorded after treatment with ≥50\% moisture content at 55 °C. However, more investigation will be needed in order to produce a more heat-stable SDS ingredient that can be used in formulations requiring additional processing.\textsuperscript{51} Optimal conditions of heat-moisture treatment to produce SDS were evaluated in waxy potato starch, reporting 41.6\% SDS content following a treatment at 25.7\% moisture content at 120 °C for 5.3 h.\textsuperscript{52} The study showed structural changes in the crystalline arrangement from B-type to a combination of B- and A-types, as well as the presence of more imperfect or heterogeneous crystallites which may be due to the broadened phase transition observed by DSC after treatments in conditions of excess of water. These alterations have been related
to the change in starch digestion rate. More recently, there has been efforts to produce SDS ingredients that can be released and hydrolysed distally, i.e. in the ileum section, in order to activate the ileal brake involved in the regulation of the gastric emptying rate; when the gastric emptying rate is low (associated with the consumption of SDS) the glycaemic response is moderate and may beneficially impact satiety.53,54

Structure-digestibility relationship

It is well-known that the above-mentioned treatments modify the original structure and the arrangement of starch components in both amorphous and crystalline lamella. Structural changes modify the starch digestion but also alters its functionality (e.g. low retrogradation, low syneresis, high water retention, etc). When applied individually or in combinations for the production of a food ingredient, these treatments generate changes in starch structure that impact its digestibility to a variable extent. For example, the slow digestion features of starch have been related to the granular X-ray diffraction pattern, chain-length distribution of amylopectin chains (short/long chains ratio), granule shape and size.55 Similarly, the information of the slow digestion (SDS) characteristic of raw cereal starches (ungelatinized) and the resistance to hydrolysis (RS) of raw tuber starches are related to the chain-length distribution.6 Amylopectin in raw cereal starches is generally characterized by its high content of short chains with high level of branching points, whilst tuber starches show more abundant long chains; however, a parabolic relationship between short/long chains ratio and SDS content was found,56 meaning that if a particular starch shows a high-level of short chains it will show high SDS content, but if long chains are present in a high proportion a high SDS content will be observed also. This issue deserves more investigation to elucidate this structure-digestibility relationship.
Structural changes produced by chemical reagents, where different functional groups are introduced, lead to reduced digestibility as digestive enzymes have lower affinity for the modified starch substrate. The impact of the chemically modified starches on the starch nutritional features in the overall diet is low due to the low concentration of modified starches used in food applications. Pyrodextrinization reactions are used to modify starch structure by introducing new glycosidic bonds (β-1-3 and β-1-6); this reaction involves incubation of dry starch with mineral acids at high temperature, yielding a soluble starch viscous solution. Pyrodextrinization can be achieved during baking of starchy foods with low moisture content, producing an increase in the RS content, an issue that should be investigated taking also into consideration the influence of the food matrix. Debranching of starch with enzymes (e.g. pullulanase) produce linear chains (amorphous areas) and residual crystalline sections rich in alpha-glucan double helices, which are organized in layered structure; this de novo arrangement of starch structure has been associated with slow digestion properties; intrinsic characteristics of starch (amylose/amylopectin ratio and chain-length distribution) play an important role in the re-arrangement of starch components after debranching. We emphasize that depending on structural characteristics of a particular native starch (e.g. X-ray diffraction pattern), debranching and subsequent aggregation and arrangement of linear chains in double helices produce crystalline structures that differ from those originally found in the native starch; this new entities cannot be hydrolysed by digestive enzymes, which results in increased RS fractions. In this sense, studies on the structure of native waxy rice starches and its relationship with digestibility showed that intrinsic characteristics, such as granule size, the presence of pores in the granule surface, crystallinity degree and chain-length
distribution of amylopectin, are important determinants of the resistance to enzymatic hydrolysis. Changes in the crystalline structure due to acid hydrolysis and subsequent esterification to produce cross-linking between short amylopectin chains originate changes in starch structure that also result in increased RS contents. Cooked rice is a common food matrix where protein and starch are the main components. In the cooking process of rice, starch suffers changes in structure that modify its digestibility; it was reported that amylopectin present in rice starch with long and intermediate chains favour a structure that resists enzymatic hydrolysis compared with rice starch with higher level of short chains.

The information generated by studies on structure-digestibility relationship can be used to propose processes to elaborate starchy foods (food matrix) with specific structure and digestibility; also, the development of starch powders with RS and SDS using different methods (single and combined) can be proposed to obtain new ingredients for foods. This issue is important due to the growing interest to produce foods with reduced and slow starch digestion features that are associated with potential use in the prevention of diverse diseases as colon cancer, type 2 diabetes, and cardiovascular problems.

**Nutraceutical effects of SDS and RS**

So far, our revision has led us to the conclusion that starch nutritional quality strongly depends on starch structural features and on their changes with processing. Now, we will discuss the potential nutraceutical effects of SDS and RS consumption, a research area of intense current interest. It is known that SDS benefits are associated with a low glycaemic index (GI), which is related to the reduction of the risk of diverse chronic degenerative diseases such as type 2 diabetes, overweight and other obesity-related disorders. Ongoing investigations evaluate the potential anti-inflammatory and oxidative stress-modulating
effects of SDS-rich regimens. However, recent reports have highlighted the importance of the particular small intestinal location where SDS is digested and its glucose is liberated and absorbed. Apparently, distal digestion can trigger an ileal brake mechanism that slows the stomach emptying rate. SDS intake has the potential to stimulate incretin hormone secretion, which is related to decreased gastric emptying, reduced food intake and increased satiety. Such effects extend nutrient delivery to the body and may decrease appetite and promote weight management; however, efforts should be focused on developing well-designed clinical studies to corroborate these results and the minimal dosages required to achieve them. Considering the complexity of the study needed, and the multiple mechanisms to contemplate, we anticipate that the first step to accomplish this would be to develop “in vitro” and “in vivo” consensual methods for SDS quantification. An in vivo method could be used for a better understanding of the SDS concept and its physiological effects, and as a tool to study food quality; however, additional questions brought forth by the EGI concept as length of measurement time, relationship between EGI and GI, and the response of the regulatory hormones during the extended period, should be primarily addressed.

The other fraction with health benefits (RS) has the ability to modulate postprandial glucose rise and insulin sensitivity, indicating that RS could be useful for managing diabetes. However, the role of RS in metabolic responses is still an issue under study since there is not sufficient evidence suggesting effects on other metabolic markers such as blood pressure and plasma lipids. Even though, the evidence from animal models indicating that RS improves a variety of metabolic features suggests that, although these benefits likely arise from a multitude of mechanisms, the gut microbiota is one of the underlying key factors. Studies in rats showed that RS may modulate the
ratio between the two major phyla present in the colonic ecosystem, Bacteroidetes and Firmicutes, by supporting the growth of beneficial bacteria\textsuperscript{77} that initiate the degradation of complex substrates, such as resistant starch particles, yielding beneficial compounds, e.g. short chain fatty acids (SCFA) (Figure 2). The effect of RS type (RS2 and RS4) on gut microbiota was evaluated in a double-blind crossover intervention in healthy young adults showing that both RS types increased Actinobacteria and Bacteroidetes phyla and decreased Firmicutes; however, RS2 increased the abundance of Ruminococcus bromii and E. rectale, and RS4 consumption was associated with increased Bifidobacterium adolescentis and Parabacteroides distasonis.\textsuperscript{83} This ability of RS to modify colonic microbiota and SCFA production is also of interest due to the physiological and metabolic impacts of these metabolites with implications on human health.\textsuperscript{84} Several in vitro (cell lines) and in vivo (rat model) studies have related SCFA to systemic effects, not only on glucose and lipid metabolism but also as key in the prevention and treatment of the metabolic syndrome, bowel disorders, and colon cancer.\textsuperscript{85-87} It was found that dietary fibre protected against colorectal cancer in a microbiota and butyrate-dependent manner.\textsuperscript{88} Also, it has been demonstrated that RS feeding produced protection against colitis-associated colorectal cancer, an action that was related to significant changes in the gut microbiota, leading to increased short-chain fatty acids production, mainly butyrate.\textsuperscript{77} However, it is perhaps unsurprising that effects of RS on SCFA production reported \textit{in vitro} and in animal models are not replicated easily in human studies. Such discrepancies are attributed to the type and dose of RS, the food matrix in which RS is incorporated, the variability of responses among individuals, and the differences in microbiota composition, aspects that should be considered for future design of studies. However, it seems clear that a RS-rich diet can lead to the establishment of a healthy gut microbiota and the
understanding of how RS interacts with gut bacteria is rapidly advancing; in this sense, further investigation may lead to classification of RS as a prebiotic. Future studies investigating the influence of RS on human health, should also consider the period of dietary changes and the fact that biological information is bidirectional, implying therefore the need of omic sciences and technologies to fully understand postprandial and longer term effects of RS consumption.

Conclusions

Initial reports indicated that starch present in foods was completely digested and absorbed, a concept that led to starchy foods restriction for people with diabetes and obesity. However, it was later discovered that different starches may be digested at different rates or not digested at all in the human small intestine. Rapidly digestible starch produces a rapid and sharp postprandial increase of glucose in the blood and it is therefore not recommended for people with the above-mentioned health problems. Slowly digestible starch (SDS) and resistant starch (RS), on the other hand, have been associated with some health benefits, such as low glycaemic and insulinaemic response, satiety and, for RS in particular, the potential prebiotic effect due to its fermentability by the colonic microbiota, leading to the production of short-chain fatty acids. Chemical, physical and enzymatic methods for producing SDS and RS with specific structure give the possibility to taylor-made both fractions with health benefits. Current investigations are directed to finely determine the location of glucose release after hydrolysis of SDS in the small intestinal lumen, and the effect of RS fermentation on differential growth of particular bacteria group in the human colon.
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REFERENCES


71. Sandberg JC, Bjorck IM and Nilsson AC, Rye-based evening meals favourably affected glucose regulation and appetite variables at the following breakfast; a randomized controlled study in healthy subjects. *PLOS ONE* **11**:e0151985 (2016).


Figure legends

Figure 1. Starch digestibility knowledge through time. Slowly digestible starch, SDS; resistant starch, RS.

Figure 2. Resistant starch effects on gut health.
Figure 2. Resistant starch effects on gut health