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Resistant Starch: A Promising Functional Food Ingredient

Revati Wanikar and Swati Kotwal

Abstract

Nowadays dietary starches are considered as a tool for maintaining good health. Recently resistant starch has received much attention because of its specific contribution to human health. Resistant starch escapes digestion in the small intestine and fermented in the colon by colonic microorganisms. Resistant starch has wide applications in varieties of food products. In the present study, types of resistant starch, their sources, physiological benefits, have been discussed briefly. This chapter focuses on factors affecting starch digestion, resistant starch content, characterization of resistant starch and various techniques employed to study their structural features.

Keywords: resistant starch, starch digestion, glycemic index, short chain fatty acids, molecular characterization, SEM

1. Introduction

The concept of resistant starch (RS) has raised interest as a source of dietary fiber. A recent recognition of resistant starch as a functional food ingredient finds application in varieties of food products. The term “Resistant Starch” was first coined by Englyst et al. in 1982 [1] and later defined formally by European Flair Concerted Action on Resistant Starch (EURESTA) as “a fraction of starch that resists digestion in the small intestine of healthy individual and passes to the large intestine where it is a substrate for bacterial fermentation” [2].

RS has potential health benefits similar to soluble fiber. The content of resistant starch in foods has considerable importance because it positively influences functioning of digestive tract, gut microflora, glycemic index, maintain blood cholesterol level and assist in the control of diabetes. These qualities of RS are attracting the attention of food industries and to understand its formation and ways to modulate its content according to the need of the human ailment [3].

History of starch and its usage by man has been extensively studied and are well documented over the years. Starch is the most significant form of carbohydrate in terms of its universality as an energy source in human diet and its applicability in varieties of food products. The understanding that starch is not completely digested and the finding that some starches are poorly digested has led to improved interest for nutritionist. Starch digestion, its impact on glucose release and its relevance to diabetes, obesity and other metabolic disorders resulted in renewed interest in intake of starchy foods. Starchy foods which release glucose slowly and over a longer period

of time after digestion are of great interest. Controlling glucose release from starchy foods has become challenge for food developers in the context of worldwide health concern. It is possible to modify the structure of starch for desired functional properties by applying various food processing [4–9].

Starch is utilized in several industrial applications due to its ability to impart broad range of functional properties to food and non food products. The new insights have increased the interest in identifying new sources of starches with distinct functional properties and their potential for processing at large scale [10, 11].

Starch is the only natural polysaccharide digested by enzymes of human gastrointestinal tract. Starch digestion starts in mouth where α -amylase in saliva breaks down starch into oligosaccharides and maltose. The bolus is then transported to the stomach where the enzyme activity is inhibited due to low pH and therefore starch does not break down until reaches to the small intestine. By the action of pancreatic α -amylase in the small intestine starch is broken down to glucose and maltose however all the starch is not hydrolysed and absorbed. Fraction of starch which escapes digestion is passed into the large intestine and fermented by intestinal microflora. Hydrolysis of starch by enzymatic digestion may be affected by digestion conditions, granule size, amylose/ amylopectin ratio and processing method of starch [12]. Starch is normally processed or cooked before being consumed by humans; hence extent of disruption of starch structure determines its susceptibility to enzymatic digestion [13].

According to in vitro digestion, starch is classified into three categories

1. **Rapidly digestible starch (RDS):** RDS is the fraction of starch that causes rapid increase in blood glucose level after ingestion. Chemically it is measured as a starch digested to glucose after 20 min of α -amylase incubation. RDS is mainly the amorphous fractions of starch and may occur in high amount in freshly cooked foods [3, 14].
2. **Slowly digestible starch (SDS):** SDS is the starch fraction that is digested slowly in the small intestine. SDS cannot be disrupted by salivary α -amylase; it is hydrolysed by pancreatic α -amylase and broken down into linear oligomers and limit dextrans. SDS measured chemically as a starch digested in 100 min of enzyme incubation. Due to slow release of glucose SDS has potential health benefits. SDS reduces risk of chronic diseases related to diet such as diabetes, obesity and other metabolic syndrome [15]. SDS occurs in raw starches with crystalline pattern of A and C type and in the retrograded starch [16].
3. **Resistant starch (RS):** RS is defined as the fraction of starch that escapes digestion in small intestine and fermented in the colon. Chemically, RS can also be defined as fraction of starch not digested after 120 min of incubation with enzymes [1, 14, 16].

2. Types of resistant starch

Depending on its resistance to digestion, RS is classified as RS1, RS2, RS3 and RS4 (Table 1).

RS1 is a physically protected starch surrounded by cell wall and other food matrix which hinders the digestibility of starch. RS1 is found in whole or partially milled seeds, cereal grains or legumes. Human gastrointestinal tract lacks the enzymes need to degrade

| Type | Description | Food sources | Resistance reduced by |
|--------|---|--|---|
| RS I | Physically protected | Whole or partially milled seeds, legumes, pasta | Milling and chewing |
| RS II | Non gelatinized granules with B-type crystallinity and are hydrolyzed slowly by α -amylase | Raw potatoes, green banana, some legumes, high amylose starch | Food processing and cooking |
| RS III | Retrograded starch | Cooked and cooled cereal products with prolong and/ or repeated moist heat treatment | Processing conditions |
| RS IV | Chemically modified starches due to cross-bonding with chemical reagents. | Some fiber drink, foods in which modified starches has been used (e.g. certain breads and cakes) | Less susceptible to <i>in vitro</i> digestibility |

Source: Nugent [17].

Table 1.
Types of resistant starch.

cellulose, hemicelluloses, lignin and other plant cell wall constituents and therefore this form of physically protected starch passes to the small intestine in intact form [18].

RS2 is a starch in a certain granular form and they are protected from digestive enzymes due to their crystalline structure. Such type of starch is mostly present in uncooked potatoes and bananas. Raw potato starch has large granule size and hence limited access to the enzymatic attack [18, 19]. The extent of starch hydrolysis is determined by the structure and size of the starch granule surface. However, No relationship has been reported between the extent of starch hydrolysis and degree of enzyme adsorption on the surface of the starch granule. Potato starches have B type crystalline pattern whereas cereal starches are characterized by A type with higher degree of crystallinity and therefore susceptible to enzymatic attack compared to potato starch. Waxy maize starch which contains 100% amylopectin with 40% crystallinity is more susceptible to digestion than high amylose maize starch with 15% crystallinity [20]. Crystallinity plays an important role in the architecture of the granules in terms of its susceptibility to enzymatic hydrolysis.

RS3 is a retrograded starch. Retrogradation occurs when starchy foods are gelatinized and cooled. Gelatinization is a process in which starch is heated in presence of water which resulted in swelling of the granule, leaching of amylose and loss of crystalline structure. Gelatinization is a complex process which starts at low temperature by swelling and continues until the granules are disrupted completely. As the temperature increases the interaction between the polymers decrease and starch granule breaks down. These structural changes take place during heating of starch in the presence of water. Extent of starch gelatinization depends on many factors such as botanical source of starch, heating rate, water content, amylose-amylopectin ratio, and processes applied to starch before gelatinization [21, 22]. Retrogradation is a process in which gelatinized starch upon cooling tends to reassociate to form more ordered structure. This re-annealing of amylose and amylopectin branches occur when gelatinized starch is stored at lower temperatures for longer period of time and thus protects from enzymatic attack [23]. Retrogradation is a property of starch, which is of particular interest in terms of nutritional significance and digestibility. Starch retrogradation was initially thought to be undesirable because of its staling effect on bread and other starchy foods, affecting shelf life and consumer acceptance. However, intensive research on retrogradation of starch over the years have shown

that it is desirable in some applications such as preparation of breakfast cereals, parboiled rice, mashed potatoes, chinese rice, because of the changes in structural, sensory and mechanical properties [23]. The most important and significant property of retrograded starch is its slow release of glucose into the bloodstream [22, 24]. Retrogradation of starch is associated with series of physical changes such as increase in viscosity, gel formation, increased degree of crystallinity with the formation of B type crystalline pattern [22]. It is an ongoing process in unstable gelatinized starch, due to rapid recrystallization in amylose polymers followed by slow recrystallization of amylopectin molecules [25].

RS4 is a chemically modified starch formed by cross linking or by adding chemical derivatives.

Recently, two components have been proposed as **RS5**. The first component is amylose-lipid-complex and second component is resistant maltodextrins [17, 26]. **RS** occurs naturally in all starchy foods and can be developed in others by combination of several processing conditions.

3. Sources of resistant starch

High amount of resistant starch is found in raw potato and unripe banana. Several studies conferred the beneficial effects of unripe banana on human health which is associated with its high **RS** content. Raw potato starch has the highest **RS** content (75%). Whole grains are rich sources of dietary fiber and resistant starch. **Table 2** provides **RS** content of some basic foods [27].

| Source | Total starch | Total dietary fiber | Resistant starch |
|---------------------|--------------|---------------------|------------------|
| Legumes | 42.6 | 36.8 | 24.6 |
| Red kidney beans | 53.3 | 33.3 | 25.4 |
| Lentils | 53.9 | 32.8 | 17.7 |
| Black-eyed peas | | | |
| Cereal grains | 55.5 | 17.0 | 18.2 |
| Barley | 77.9 | 19.6 | 25.2 |
| Corn | 50.0 | 17.0 | 13.6 |
| Wheat | 95.1 | 1.5 | 14.1 |
| White rice | | | |
| Cereal products | 67.4 | n/a | 1.4 |
| Crisp bread | 46.7 | n/a | 1.9 |
| White bread | 67.0 | n/a | 1.2 |
| Puffed wheat cereal | | | |

Source: J. Lunn et al. [27].

Table 2.
RS content of some basic foods (g/100 g).

4. Nutritional and health impact of resistant starch

4.1 RS as a prebiotic agent

‘Prebiotics’ are food ingredients that help support growth of probiotic bacteria. Prebiotics are considered as nondigestible carbohydrates such as resistant starch

which ferment in the colon by gut microflora. Essentially they stimulate activity of good bacteria such as *Lactobacilli*, *Bifidobacteria* and *Staphylococci* and confer benefits upon host health [22].

4.2 Prevention of colon cancer

Resistant starch escapes digestion in the small intestine and is fermented in the large intestine resulting in the production of short chain fatty acids (SCFA), some gases like methane, hydrogen and carbon dioxide and organic acid (e.g. lactic acid) [28]. SCFA include acetate, propionate and butyrate. A number of studies have indicated the benefits of resistant starch as it produces SCFA, as compared to dietary fiber, especially butyrate production is more. Butyrate is the main energy substrate for colonocytes and several in vitro studies have shown that butyrate inhibits malignant transformation of cells by arresting one of the phases of cell cycle (G1) [23, 25]. More butyrate production is associated with lower incidence of colon cancer [29, 30]. **Table 3** presents data on SCFA produced by the fermentation of some foods in the large intestine.

4.3 Hypoglycaemic effects

Foods containing high resistant starch reduce the rate of digestion. Slow rate of digestion has implications for the use of RS in controlled glucose release applications. Starch digestion and concurrent changes in blood glucose levels are largely dependent on its rate of hydrolysis by α -amylase and extent of digestion. From the health point of view, the starches that are less susceptible to α -amylase attack score high as they bring about less change in post prandial glucose level and more starch enters the colon undigested. RS consumption is associated with reduced post prandial glycemic and insulinemic response. Therefore RS can help in the treatment of diabetes, obesity and in weight management [30].

4.4 Hypocholesterolemic effects

Based on the studies in rats, RS is shown to affect lipid metabolism where reductions in measures of lipid metabolism is observed (total lipids, total cholesterol, LDL, HDL, VLDL) [30].

| Substrate | SCFA (%) | | | References |
|------------------|----------|------------|----------|------------|
| | Acetate | Propionate | Butyrate | |
| Resistant starch | 41 | 21 | 38 | 35 |
| Oat bran | 57 | 21 | 23 | 35 |
| Wheat bran | 57 | 15 | 19 | 35 |
| Cellulose | 61 | 20 | 19 | 35 |
| Guar gum | 59 | 26 | 11 | 35 |
| Pectin | 75 | 14 | 9 | 35 |

Source: A. Sharma et al. [31].

Table 3.
Percentage of total SCFA produced by various substrates.

4.5 Inhibition of fat accumulation

Various studies examined that high RS meals may increase the use of fat stores as a result of reduction in insulin secretions. High RS meals imparted less satiety than low RS meals whereas in another study on human volunteers, high RS meal caused greater satiety [32]. Keenan et.al reported in their study that incorporating RS in diet may increase the gut hormones that are effective in reducing energy intake. This may be an effective approach for the treatment of obesity [33].

4.6 RS as a functional ingredient

The functional properties of resistant starch such as swelling, viscosity, gel consistency, water holding capacity make it useful in variety of food applications. Low water holding capacity of RS makes it a functional ingredient which provides good handling in processing, crispness, expansion and improved texture of food products [14, 19]. Hi-maize is the first commercial RS introduced in the market in 1993 in Australia. The other sources of commercial RS 3 are CrystaLean, Novelose and Actistar which are highly retrograded starches. Fibersym is a chemically modified RS 4 product [14]. RS may find applications in varieties of food products such as bakery products [34, 35], pastas and puddings [14, 36, 37], yoghurt, cheese, icecreams [19, 38, 39]. RS incorporated biscuits has been investigated and reported that incorporating RS in foods have potential to develop fiber rich products without changing their general properties. RS can also be used as thickening agent and substituted fat in imitation cheese and many other products where insoluble fiber is desirable conferring the benefits of RS as a functional fiber. Bread and pasta are the most widely consumed starch based products. RS as a food ingredient is increasingly important as resistant starch has low calorific value (8 kJ/g) compared to fully digestible starch (15 kJ/g) [18].

5. Factors affecting starch digestibility and resistant starch content

The structural changes of starch during processing are the major determinants of starch functional properties for food processing, during digestion and in industrial applications [25, 40]. Wide range of techniques has been used for processing the food materials which involve chemical and hydrothermal treatments. The processing methods are reported to influence the nutritional characteristics of foods. Roasting and cooking without pressure are some of the major processes used in household whereas domestic storage is also a widely used method now-a-days. Processing methods are the major determinants of starch digestibility and amount of starches reaching the colon [41]. Gelatinization and retrogradation are important properties of starch that determine its functionality, quality, acceptability and nutritional value [22]. Several inherent properties of starch influence the formation of RS and starch digestibility are discussed below.

5.1 Granule morphology

Size and shape of starch granule is influenced by botanical origin. Several studies have indicated negative relationship between large granule size of wheat, barley, and potato and starch digestibility. The rate of starch hydrolysis is increased by decreasing the size of the granule. This was observed among starches with different botanical origin [15]. Smaller granules have the higher susceptibility to enzyme binding [42].

5.2 Surface of granules

Starch hydrolysis is also dependent on the shape of the granules which varies from spherical to polyhedral. The molecular association of starch granules may reduce the binding of amylase to granule surface [43].

The surface characteristics such as pin holes, equatorial grooves, indentations and small nodules have an impact on starch digestibility [12] smooth surface of potato and high amylose starches with few pits and pores explain the starch resistance to amylases [42, 43].

5.3 Molecular structure and crystallinity

The different crystalline patterns of starches such as A, B and C differ in their packing of double helical structures of amylopectin molecules thereby influencing their hydrolysis [44] It is reported that B type crystalline starches are more resistant to amylolytic attack than A type. Amylase attack also depends on linear chain length of amylopectin molecule which forms the helices. The longer chains are more resistant to enzymatic hydrolysis due to more stable helices [12, 15]. The hydrolysis starts earlier in the amorphous region of C type crystalline starches. Additionally, the crystalline distribution in granules has an impact on digestibility. Higher resistance was observed in starches with higher amount of double helices. This may be attributed to the resistance of high amylose native starches, which are less crystalline than native starches with high crystallinity.

5.4 Amylose amylopectin ratio

There is a positive correlation between amylose content and resistant starch formation. The linear amylose chains are bound to each other by hydrogen bonds which make them less accessible to hydrolysis [12]. The high proportion of amylopectin molecule in starch granule makes the larger surface area and therefore a molecule becomes more accessible to amylolytic attack. Starch gelatinization is difficult in high amylose starches and is more susceptible to retrogradation [36]. The *in vitro* and *in vivo* starch digestibility of high amylose starches were reported to be lower than normal starches [45].

5.5 Interaction of starch with other components

Food matrixes such as proteins and lipids play significant role during processing and affect the starch digestibility.

5.5.1 Lipids

Lipids are associated with starch granules. The free fatty acids and phospholipids are complexed with amylose and make the starch resistant to digestion. The lipids are usually present on the surface of the granules and reduce the binding of enzymes. The enzymatic digestibility is also reduced by addition of lauric, palmitic and oleic acid [43, 46].

5.5.2 Proteins

The surface proteins influences enzyme binding and limit the rate of hydrolysis. The starch from pulses is hard to digest due to interaction with proteins and presence of protective network around the granule [12].

5.5.3 Dietary fiber

Gaur and xanthan gums are some of the dietary fibers which affect the digestibility due to their high viscosity which slows down the movement and absorption of digestion products in the small intestine [14].

5.5.4 Ions

Phosphorous as phosphate monoesters and phospholipids significantly affect the starch properties. The tendency of phospholipids to form complexes with amylose and amylopectin makes the starch less susceptible to enzyme hydrolysis. Calcium and potassium ions reported to decrease RS yield [47].

6. Techniques used to study morphological, molecular and thermal characteristics of resistant starch

Understanding the molecular characteristics of starches to study the functional behavior and their suitability and applicability in various food industries is of great importance.

Thermal property is an important functional property of starch that varies with respect to the macromolecular composition (amylose and amylopectin ratio), double helical structure of amylopectin (chain length, branching, and degree of polymerization) and granule architecture (amorphous to crystalline ratio), granule morphology and size distribution. Differential scanning calorimetry (DSC) is the widely used technique to study thermal behavior of starches as well as other polymers. DSC can characterize modifications in starches, high amylose starches and waxy maize starches as well [48].

Spectroscopic techniques can provide appropriate information about the native as well as modified starches and their structural features. It also provides information of structural changes during gelatinisation and retrogradation.

The infrared (IR) spectroscopy can detect the molecular bond vibrations (especially C—O and C—C bonds) which yield both qualitative and quantitative information, such as that on the amorphous and crystalline regions of the starch granule [49]. Using FT-IR technique it was also observed that the high amylose maize and potato starches (RS2) exhibited greater level of ordered structure in the external region than wheat, maize or waxy maize starches. Due to retrogradation during storage, conformational changes in starches can be monitored and the intensity changes of conformational-sensitive bands in the $1300\text{--}800\text{ cm}^{-1}$ region could be observed [23].

Scanning electron microscopy technique is generally used to provide topographic features of RS. Differences in granule morphology of starches can also be detected using SEM [48, 50]. SEM images of native versus resistant starches formed from different processing techniques are shown in **Figure 1**. Pinholes on the surface of the starch granules were observed in native starches isolated from millets. RS from cooked samples showed irregular and uneven surface zone. RS from retrograded starches showed fibrous, compact and less smooth structures [51].

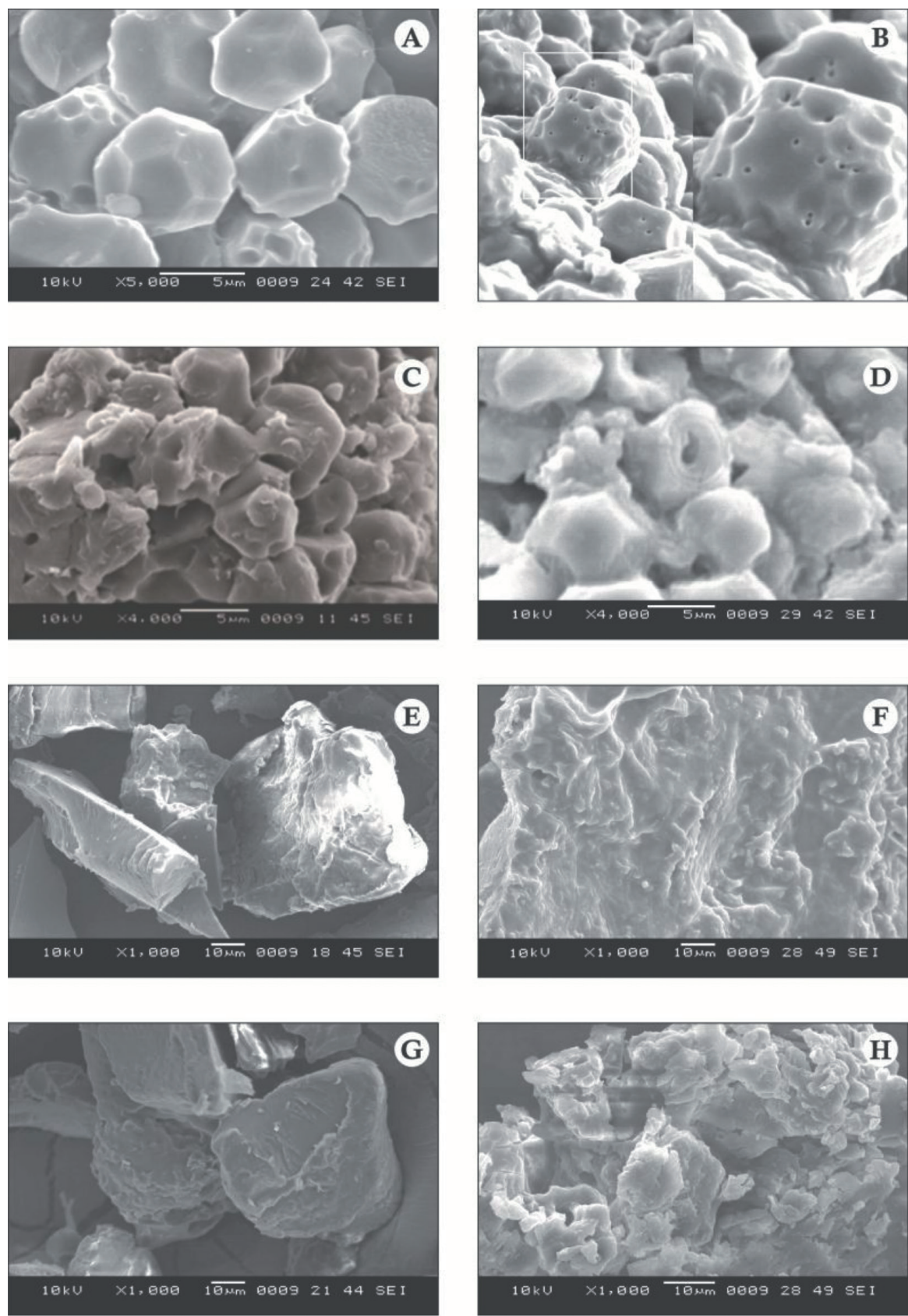


Figure 1.
SEM image of native versus resistant starches. Note: (A) TS native, (B) RS native, (C) TS roasted, (D) RS roasted, (E) TS cooked, (F) RS cooked, (G) TS cooked and stored at -20°C for 30 days, (H) RS cooked and stored at -20°C for 30 days. TS: total starch; and RS: resistant starch. Source: R. Wanikar [51].

X-ray diffraction can be applied to investigate different pattern of crystalline Structure and crystallinity of starch obtained from various botanical sources. XRD generally detects the regularly repeating ordering of helices and thereby reflecting the three-dimensional order of crystalline structure of starch [52].

There is an increased awareness in consumers for health and diet which has led enormous research on resistant starch, its content in foods and structural characterization. When combined the information generated from the above techniques can provide comprehensive analysis of structural characteristics of resistant starch, as well as changes occur during the formation of RS when compared with the structure of their native starches.

7. Conclusion

Resistant starch is not accessible to digestive enzymes. This undigested starch fraction is of particular significance to human health as it lowers the calorific value of food and therefore provides a means to use as a potential food ingredient. The content of resistant starch can be increased by various food processing. Consumer's awareness about health and food is one of the reasons for increased popularity of extensive research on resistant starch and their health impact. Structural characterization of RS by using different techniques and their relationship needs a deeper understanding. Further studies are needed to clarify the relationship between physiological effects and molecular characterization of RS. In vitro RS fermentation and colon cancer incidence is an important aspect for further study.

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