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Resistant Starch: A Potential Impact on Human Health

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Dietary starches are important sources of energy for many human societies and it is clear that they can also make quite specific contributions to health. Resistant starch has received much attention for both its potential health benefits (similar to soluble fibre) and functional properties. Resistant starch (RS) encompasses forms of starch, which are not digested in the small intestine by enzymes but fermented in the large intestine. It occurs for various reasons including chemical structure, cooking of food, chemical modification, and food mastication. Human colonic bacteria ferment RS and nonstarch polysaccharides (NSP; major components of dietary fiber) to short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. SCFA stimulate colonic blood flow and fluid and electrolyte uptake. Resistant starch positively influences the functioning of the digestive tract, microbial flora, the blood cholesterol level, the glycemic index and assists in the control of diabetes. Apart from the potential health benefits of resistant starch, another positive advantage is its lower impact on the sensory properties of food compared with traditional sources of fibre, as whole grains, fruits or bran.

Introduction

Starch, which is the major dietary source of carbohydrates, is the most abundant storage polysaccharide in plants, and occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses, and tubers (Ellis and others, 1998). The relatively recent recognition of incomplete digestion and absorption of starch in the small intestine as a normal phenomenon has raised interest in non digestible starch fractions (Cummings and Englyst, 1991; Englyst and others, 1992). These are called "resistant starch-es," and extensive studies have shown them to have physiological functions similar to those of dietary fiber (Asp, 1994; Eerlingen and Delcour, 1995). RS has been defined as the fraction of starch, which escapes digestion in the small intestine, and may be digested in the large intestine (Englyst, Kingman and Cummings, 1992).

This is similar to the traditional definition, except for the qualification that fiber is non-starch in origin. RS offers an exciting new potential as a food ingredient. It has been shown to possess physiological benefits similar to soluble fibers, and, in addition, to be used as a mechanism for sustained glucose release.

Starch and its classification

Starch is the dominant carbohydrate reserve material of higher plants, being found in leaf chloroplasts and in the amyloplasts of storage organs such as seeds and tubers. Biosynthesis of starch granules takes place primarily in the amyloplasts.

Starches isolated from different botanical sources display characteristic granule morphology. Starch granules vary in shape (spherical, oval, polygonal, disk, elongated and kidney shapes), in size (1 µm-100 µm in diameter), in size distribution (uni or bimodal), in association of individual (simple) or granule clusters (compound) and in composition (α-glucan, lipid, moisture, protein and mineral content). Some different starch granules can be seen in Figure 1. Normal and waxy maize starches (Figure 1a and 1b) are spherical and polygonal in shape. Wheat starch has bimodal size distribution (Figure 1c). The large granules have a disk shape, whereas the small granules have a spherical shape. High amylose maize starches (Figure 1d) have elongated, filamentous granules, in addition to polygonal and spherical granules. The greater the amylose content of the starch, the greater the number of filamentous granules found in the high amylose maize starch (Jane, 2009 and Tester *et al*., 2004). Starch granules are composed of two types of *alpha*-glucan, amylose and amylopectin, which represent approximately 98-99 % of the dry weight. The ratio of the two polysaccharides varies according to the botanical origin of the starch; normal starches contain 70-80 % amylopectin and 20-30 % amylose (Jane, 2009 and Tester *et al*., 2004). In mutant lines of diploid species originating from crops such as maize, starches can be obtained with amylose contents in the range of 0% (waxy maize) to 84% (amylomaize) (Matveev *et al*., 2001).

Amylose and amylopectin have different structures and properties; however, both molecules are composed of a number of monosaccharides (glucose) linked together with *alpha*-1-4 and/or *alpha*-1-6 linkages. Amylose is a mainly linear polymer consisting of long chains of *alpha*-1-4-linked glucose units.

Amylopectin is a much larger molecule than amylose with a molecular weight of $1x10^7$–$1x10^9$ and a heavily branched structure built from about 95 % *alpha*-1-4 and 5 % *alpha*-1-6 linkages.

Classification of starches based on their nutritional properties

For nutritional purposes, starch can be classified into three categories by the Englyst test (Berry, 1986 and Englyst *et al*., 1992), depending on their rate and extent of digestion; these include rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). The main enzymes, which take part in starch hydrolysis, are amylases and amyloglucosidases resulting glucose, maltose and dextrins liberation during the digestion (Annison and Topping, 1994).

RDS is the fraction of starch granules that cause a rapid increase in blood glucose concentration after ingestion of carbohydrates. This fraction of starch *in vitro* is defined as the amount of starch digested in the first 20 min of a standard digestion reaction mixture (Englyst *et al*., 1992). Although RDS is defined by experimental analysis of digestion *in vitro*, the rate of starch conversion to sugar follows similar kinetics in the human digestive system (Dona *et al*., 2010).

SDS is the fraction of starch that is digested slowly but completely in the human small intestine (Dona *et al*., 2010). SDS is defined as the starch that is digested after the RDS but in no longer than 120 min under standard conditions of substrate and enzyme concentration (Englyst *et al*., 1992). The potential health benefits of SDS *in vivo* include stable glucose metabolism, diabetes management, mental performance, and satiety (Lehmann and Robin, 2007). Mostly physically inaccessible amorphous starches, raw starches with A-type or C-type crystalline pattern and B-type starches either in granule form or retrograded form belong to this type.

The fraction of starch that escapes digestion in the small intestine, and cannot be digested within 120 min, is defined as RS (Dona *et al*., 2010). The term of resistant starch derives from Englyst *et al*., (1982). Later, it has been defined formally by the European Flair Concerted Action on Resistant Starch (EURESTA) as the starch or products of starch degradation that escapes digestion in the human small intestine of healthy individuals and may be completely or partially fermented in the large intestine as a substrate for the colonic microflora acting as a prebiotic material (Faraj *et al*., 2004).

Resistant starch has been classified into four general subtypes (Figure 2) called RS1, RS2, RS3 and RS4 (Asp *et al*., 1996; Englyst *et al*., 1992; Fuentes-Zaragoza *et al*., 2010; Nugent, 2005 and Sajilata *et al*., 2006).

RS1 (Figure 2a): It has a compact molecular structure which limits the accessibility of digestive enzymes. This starch is entrapped within whole or partly milled grains or seeds and tubers (Fuentes-Zaragoza *et al*., 2010 andHaralampu, 2000). It is measured chemically as the difference between the glucose released by the enzyme digestion of a homogenized food sample and that released from a non homogenized sample. RS1 is heat stable in most normal cooking operations, which enables its use as an ingredient in a wide variety of conventional foods (Sajilata *et al*., 2006).

RS2 (Figure 2b): RS2 are native, uncooked granules of starch, such as raw potato, banana and high amylose maize starches, whose crystallinity makes them poorly susceptible to hydrolysis. They are protected from digestion by the conformation or structure of the starch granule. This compact structure (tightly packed in a radial pattern and is relatively dehydrated) limits the accessibility of digestive enzymes, and accounts for the resistant nature of RS2. A particular type of RS2 is unique as it retains its structure and resistance even during the processing and preparation of many foods; this RS2 is called high amylose maize starch. RS2 is measured chemically as the difference between the glucose released by the enzyme digestion of a boiled homogenized food sample and that from an unboiled, non homogenized food sample (Fuentes-Zaragoza *et al*., 2010; Nugent, 2005 and Sajilata *et al*., 2006).

RS3 (Figure 2c): RS3 refers to non-granular starch-derived materials that resist digestion. Starch granules are disrupted by heating in an excess of water in a process commonly known as gelatinisation, which renders the molecules fully accessible to digestive enzymes. However, if these starch gels are then cooled (retrogradation), they form starch crystals that are resistant to enzymes digestion. It may be formed in cooked foods that are kept at low or room temperature. Therefore, most moisture heat-treated foods contain some RS3. It is found in small quantities (approximately 5%) in foods such as corn-flakes or cooked and cooled potatoes. RS3 can be divided into two subtypes: RS3a (IIIa) containing crystalline amylopectin and RS3b (IIIb) having a partially crystallized amylose network

(Themeier *et al*., 2005). It is measured chemically as the fraction, which resists both dispersion by boiling and enzyme digestion. RS3 is of particular interest, because of its thermal stability. This allows it to be stable in most normal cooking operations, and enables its use as an ingredient in a wide variety of conventional foods. Food processing, which involves heat and moisture, in most cases destroys RS1 and RS2 but may form RS3 (Fuentes-Zaragoza *et al*., 2010; Haralampu., 2000 and Sajilata *et al*., 2006).

RS4 (Figure 2d): RS4 describes a group of starches that have been chemically modified (conversion, substitution, or cross-linking) and include starches which have been etherised, esterified or cross-bonded with chemicals in such a manner as to decrease their digestibility. RS4 may be further subdivided into four subcategories according to their solubility in water and the experimental methods by which they can be analyzed. The level of resistance depends on the starch base and the modification reaction (Fuentes-Zaragoza *et al*., 2010; Nugent, 2005 and Sajilata *et al*., 2006).

Health properties of resistant starches

RS has received much attention for both its potential health benefits and functional properties (Sajilata *et al*., 2006). Resistant starch is one of the most abundant dietary sources of non-digestible carbohydrates (Nugent, 2005) and could be as important as NSP (non-starch polysaccharides) in promoting large bowel health and preventing bowel inflammatory diseases (IBD) and colorectal cancer (CRC) (Topping *et al*., 2003) but has a smaller impact on lipid and glucose metabolism (Nugent, 2005). A number of physiological effects have been ascribed to RS, which have been proved to be beneficial for health (Sajilata *et al*., 2006). The physiological properties of resistant starch can vary widely depending on the study design and differences in the source, type and dose of resistant starch consumed (Buttriss and stokes, 2008).

It is possible that modern processing and food consumption practices have led to lower RS consumption, which could contribute to the rise in serious large bowel disease in affluent countries. This offers opportunities for the development of new cereal cultivars and starch-based ingredients for food products that can improve public health. These products can also be applied clinically (Topping *et al*., 2003). There is also increasing interest in using RS to lower the energy value and available carbohydrate content of foods. RS can also be used to enhance the fiber content of foods and is under investigation regarding its potential to accelerate the onset of satiation and to lower the glycaemia response. The potential of RS to enhance colonic health, and to act as a vehicle to increase the total dietary fiber content of foodstuffs, particularly those which are low in energy and/or in total carbohydrate content (Table I).

Prevention of colonic cancer and the role of SCFA

RS, by escaping digestion in the small intestine, is fermented in the large intestine resulting in the production of such fermentation products as carbon dioxide, methane, hydrogen, organic acids (e.g. lactic acid) and SCFA. SCFA produced include butyrate, acetate and propionate, and it is thought that these SCFA in particular mediate the effects of RS, rather than RS exerting a physical bulking effect (Nugent., 2005). As butyrate is the main energy substrate for large intestinal epithelial cells (colonocytes) and inhibits the malignant transformation of such cells

*in vitro*by arresting one of the phase of cell cycle (G1); this makes easily fermentable RS fractions especially interesting in preventing colonic cancer. The fermentation behaviour of RS in vivo was evaluated under a simulated environment using microbial flora extracted from fresh faeces of healthy adults and babies of humans in vitro. The results showed that the concentration of short-chain fatty acids, especially butyric acid, in the fermented product gradually increased with increased fermentation time and RS content. The production model of acids demonstrated that maize RS prepared by the enzymatic method can be a promising ingredient of functional foods (Zhang *et al*., 2012).

Significant changes in faecal pH and bulking as well as greater production of SCFA in the cecum of rats fed RS preparations have been reported. A low (acid) pH in combination with high concentrations of SCFA is thought to prevent the overgrowth of pH-sensitive pathogenic bacteria. In most human studies, increased faecal excretion and/or faecal concentrations of SCFA were reported following supplementation with RS (Nugent, 2005 and Sajilata *et al*., 2006). Recently, it was shown that RS dose-dependently suppressed the formation of colonic aberrant crypt foci (precursor lesions of colorectal cancer) only when it was present during the promotion phase to a genotoxic carcinogen in the middle and distal colon, suggesting that administration of RS may retard growth and/or the development of neoplastic lesions in the colon. Therefore, colon tumorigenesis may be highly sensitive to dietary intervention (Fuentes-Zaragoza *et al*., 2010 and Liu and Xu, 2008).

Hypoglycaemic effects

The GI of starchy foods may depend upon various factors such as the amylose/amylopectin ratio, the native environment of the starch granule, gelatinization of starch, water content and baking temperature of the processed foods. Thus, the factors affecting the GI values are in accordance with those of RS formation. Intake of maize (25 and 50 grams of HI-MAIZE RS2 resistant starch) has been shown to improve insulin sensitivity in overweight and obese men by 54% and 73%, respectively. In this study, 11 overweight men and 22 overweight women consumed HI-MAIZE for four weeks. However, no significant effects in women were observed (Maki *et al*., 2012).

Foods containing RS reduce the rate of digestion. The slow digestion of RS has implications for its use in controlled glucose release applications and therefore, a lowered insulin response and greater access to the use of stored fat can be expected and, potentially, a muted generation of hunger signals. Therefore, RS can help possibly in the treatment of obesity and in weight management (Cummings *et al*., 2004 and Nugent, 2005). Next to the prevention of obesity, RS can also play a protective role in coronary diseases, gastrointestinal disorders and inflammatory bowel diseases (Tungland and Meyer, 2002).

RS as a prebiotic agent

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or more bacteria (probiotics) in the gastrointestinal tract and thereby exert a health-promoting effect (Scholz-Ahrens *et al;* 2007 and Roberfroid, 2000). Since RS almost entirely passes the small intestine, it can behave as a substrate for growth of the probiotic microorganisms. *In vitro*studies have shown that RS-supplemented diet may significantly increase populations of *Lactobacilli*, *Bifidobacteria*, *Staphylococci* and *Streptococci*, decrease the Enterobacteria population, and alter microbial enzyme metabolism in the colon (Perera *et al*., 2010).

Hypocholesteromelic effects

RS appears to particularly affect lipid metabolism, as seen from studies in rats, where reductions in a number of measures of lipid metabolism have been observed. These include total lipids, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides and triglyceride-rich lipoproteins(Nugent, 2005).

For a balanced view of the effect on RS on health, it is important to note that the consumption of high amounts of RS may have some negative effects on gastrointestinal performance. These include bloating, borborygmi (noise due to gas movement in the intestine), flatulence, colic and watery faeces. Overall, the benefits of RS consumption are considered to outweigh any gastrointestinal discomfort (Perera *et al*., 2010).

Inhibition of fat accumulation

A number of authors have examined the potential of RS to modify fat oxidation (Nugent, 2005) and various studies (Sharma *et al*., 2008) have examined its potential as satiety agent and also an ingredient for weight management (Mikušová *et al.*, 2009), although the results are still not conclusive. It is proposed that eating a diet rich in RS may increase the mobilization and use of fat stores as a direct result of a reduction in insulin secretion (Tapsell., 2004). Keenan *et al*., (2006) reported that the use of resistant starch in the diet as a bioactive functional food component is a natural, endogenous way to increase gut hormones that are effective in reducing energy intake, so may be an effective natural approach to the treatment of obesity.

Absorption of minerals

Resistant starch enhances the ileal absorption of a number of minerals in rats and humans. Lopez *et al*., (2001) and Younes *et al*., (1995) reported an increased absorption of calcium, magnesium, zinc, iron and copper in rats fed RS-rich diets. In humans, these effects appear to be limited to calcium (Trinidad *et al*., 2006 and Coudray *et al*., 1997). RS could have a positive effect on intestinal calcium and iron absorption. A study to compare the apparent intestinal absorption of calcium, phosphorus, iron, and zinc in the presence of either resistant or digestible starch showed that a meal containing 16.4% RS resulted in a greater apparent absorption of calcium and iron compared with completely digestible starch (Morita *et al.*, 1999).

Factors affecting the resistant starch content and the enzymatic hydrolysis of starches

Several factors influence the formation of RS, the resistance of starches and the hydrolysis of starches itself which are discussed in detail below:

Granule morphology (size, shape)

Granule morphology such as size and shape of starch granules is influenced by the botanic origin. There are several studies (Kaur *et al*., 2007b., Lindeboom *et al*., 2004 and Singh *et al*., 2010) in which negative relationship was detected between large size granules of wheat, barley and potato starches and starch digestibility. Among starches with different botanic origin it was also observed (Lehmann and Robin., 2007) that the rate of hydrolysis increased by decreasing the granule size (in the order of wheat starch > maize starch > pea starch > potato starch).

The higher susceptibility of the smaller granules can be attributed to the bigger specific surface area which may increase the extent of enzyme binding (Tester *et al*., 2006). Next to the size of granules, great significance has to be attributed to the granule size distribution (Tester *et al*., 2006).

The other morphological parameter i.e. shape also determines the starch hydrolysis. The shape of granules varies from very spherical to polyhedral thus affecting the specific surface area significantly (Singh *et al*., 2010). Additionally, the molecular association of granules reduces the capacity for amylases to bind to granule surfaces thus decreasing the specific surface area (Singh *et al*., 2010., Tester *et al*., 2006 and Zhang and Oates, 1999).

Surface of the starch granule

The surface characteristics of the starch granules have been observed to influence their enzymatic digestion. Pin holes, equatorial grooves and small nodules have an impact on the entry of the amylases to digestion (Singh *et al*., 2010). Other starches such as potato and high amylose starches have smoother surface and fewer pits or pores which can explain the resistance of these starches to amylases (Lehmann and Robin, 2007 and Tester *et al*., 2006).

Amylose-amylopectin ratio

A higher content of amylose lowers the digestibility of starch due to positive correlation between amylose content and formation of RS (Sajilata *et al*., 2006). The amylopectin is a much larger molecule than amylose; therefore, it has a much larger surface area per molecule than amylose which makes it a preferable substrate for amylolytic attack. Furthermore, the glucose chains of amylose starch are more bound to each other by hydrogen bonds making them less available for hydrolysis (Singh *et al*., 2010). The greater the content of amylose is, the more difficult the starch is to gelatinise (Gelencsér, 2009) and the more susceptible to retrogradation (Topping *et al*., 2003). Additionally, the *in vitro* and *in vivo* digestibility of high amylose starch containing products was lower than that of the control products without these starches (Gelencsér., 2009). Ao *et al*., (2007) observed that the chain length unit of amylopectin showed correlation with the digestibility.

Moreover, the chain length, the degree of polymerization of amylose and amylopectin molecules and the branch density also have an impact on RS content (Ao *et al*., 2007 and Perera *et al*., 2010). The rate of starch hydrolysis is controlled by mass transfer rate (influenced by molecular weight distribution, degree of polymerization, content of 1,6 branching bonds of the starches) and the effects of the starch structure are dependent on the substrate concentration (Singh *et al*., 2010).

Retrogradation of amylose

The rate and extent to which starch may retrograde after gelatinisation essentially depends on the amount of amylose present. Repeated autoclaving of starch may generate up to 10% RS. The retrogradation of amylose was identified as the main mechanism for the formation of RS in processed foods (Sajilata *et al*., 2006).

Interactions of starch with other components

Some molecules naturally occurring in food sources may have inhibitory effects on starch hydrolysis. Additionally, the other constituents of the food matrix, such as proteins, lipids and polysaccharides can play a significant role during processing thereby affecting the physicochemical characteristics of foods and the digestibility of starches.

Lipids

The most important non-starch components associated with starch granule may be the lipids (1-14 g/kg starch). The lipids (usually free fatty acids and phospholipids) are complexed with amylose which makes the amylose chains much less readily accessible to the active site of *alpha*-amylase. They can be usually found on the surface of the granule thus reducing contact between enzyme and substrate (Svihus *et al*., 2005 and Tester *et al*., 2006).

The addition of lauric, myristic, palmitic and oleic acids can reduce the enzymatic digestibility probably due to the formation of inclusion complexes of amylose with small hydrophobic molecules. The enzymatic resistance of complexes increases with increasing amylose degree of polymerization, lipid chain length and complexation temperature (Singh *et al*., 2010).

Proteins

The surface proteins (3 g or less g/kg starch) may also limit the rate of enzymatic hydrolysis by blocking the adsorption sites and therefore influences enzyme binding (Singh *et al*., 2010 and Tester *et al*., 2006). The pulse starches are lower digestible due to their interaction with proteins which form a protective network around the granule (Lehmann andRobin, 2007). Additionally, the presence of food proteins may influence the rate of starch digestion. The physical barrier created by the protein network (disulfide-linked polymers) in cereals may account for decreased glycaemic response and reduced rate of digestion (Sajilata *et al*., 2006 and Singh *et al*., 2010).

Dietary fibre

Some dietary fibres (guar and xanthan gums) can slower the rate of glucose release through their high viscosity which slows down the absorption of digested products in the small intestine (Singh *et al*., 2010). Other fibres (cellulose, lignin) have only minimal effects on RS yields (Sajilata *et al*., 2006).

Table.1 Health properties of resistant starches (Fuentes-Zaragoza *et al*., 2010 and Nugent, 2005)

Potential physiological effects	**Conditions where there may be a protective effect**
Control of glycaemic and insulinaemic responses	Diabetes, impaired glucose and insulin responses, the metabolic syndrome
Improved bowel health	Colorectal cancer, ulcerative colitis, inflammatory bowel disease, diverticulitis, constipation
Improved blood lipid profile	Cardiovascular disease, lipid metabolism, the metabolic syndrome
Prebiotic and culture protagonist	Colonic health
Increased satiety and reduced energy intake	Obesity
Increased micronutrient absorption	Enhanced mineral absorption, osteoporosis
Adjunct to oral rehydration therapies	Treatment of cholera, chronic diarrhoea
Synergistic interactions with other dietary components, e.g. dietary fibres, proteins, lipids	Improved metabolic control and enhanced bowel health

Fig.1 Different starch granules: (a) normal maize; (b) waxy maize; (c) wheat; (d) high amylose maize (Jane, 2009)

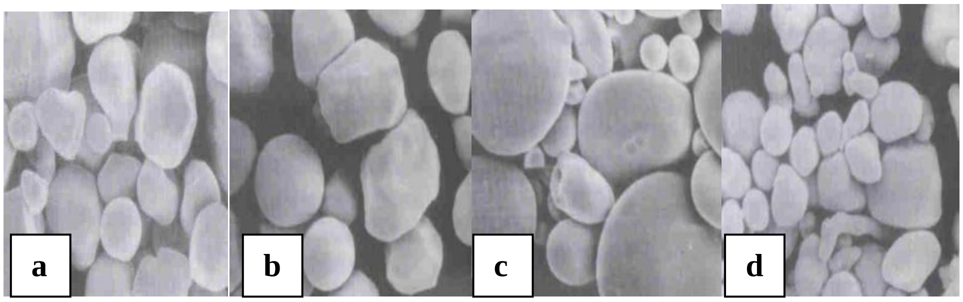
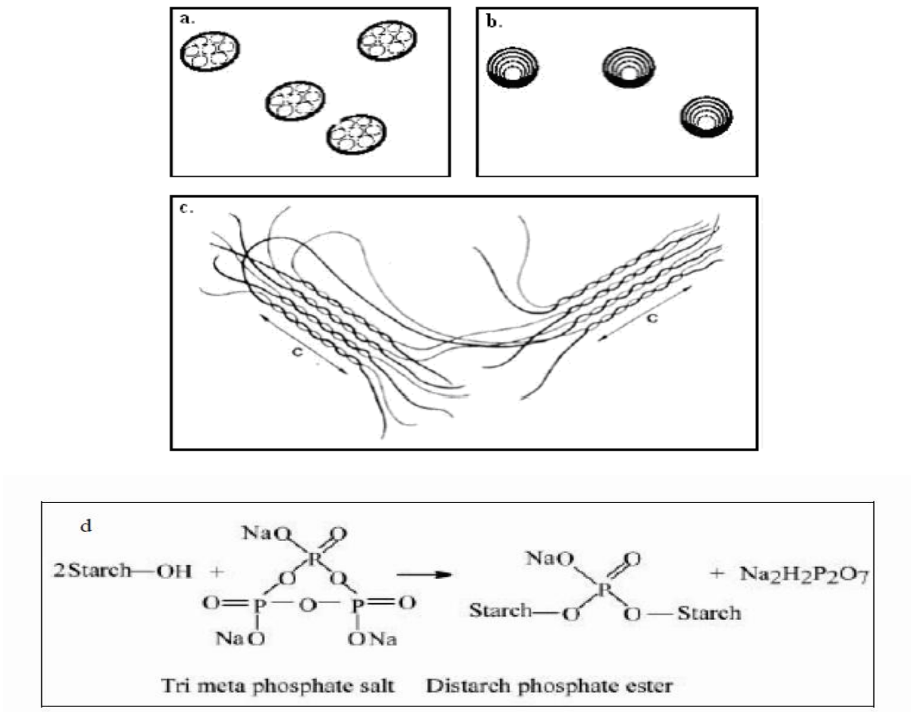


Fig.2 Types of resistant starches: (a) RS1; (b) RS2; (c) RS3; (d) RS4 (Sajilata *et al*., 2006)



Ions

Phosphorus naturally presents in starches as phosphate monoesters and phospholipids and significantly affects the functional properties of starches. Phospholipids have a tendency to form a complex with amylose and long branched chains of amylopectin (Singh *et al*., 2010). Moreover, the phosphorylated starch is less susceptible by enzymes (Tester *et al*., 2006). Additionally, Escarpa *et al*., (1997) showed investigating potato starch gels that calcium and potassium constituents cause decrease in the yields of RS probably due to the prevention of formation of hydrogen bonds between amylose and amylopectin chains caused by the absorption of these ions.

Enzyme inhibitors and other components

A wide variety of food crops such as wheat, rye, triticale and sorghum (not in rice, barley and maize) contain amylase inhibitors which may inhibit the pancreatic *alpha*-amylase (Singh *et al*., 2010). Additionally, the high concentration of anti-nutrients and other components such as phytic acid, lectins, polyphenols, sugars and hydrolysis products especially maltose and maltotriose may also play a role in starch digestion (Asp *et al*., 1996; Sajilata *et al*., 2006 and Singh *et al*., 2010).

It can be concluded that the resistant starch plays an important role in human nutrition due to its physiological and functional properties. The consumption of resistant starch and RS enriched products provide healthier life expectation and food with appropriate sensory properties. The question of resistance; however, is very complex; therefore, the investigations of the RS is especially important. So far there is no information available regarding starch digestibility characteristics of Indian maize genotypes. Therefore the present study aims at identifying the elite Indian maize genotypes having higher RS content.

In conclusion, RS has received much attention for both its potential healthbenefits and functional properties. As a functional fiber, its fineparticles and bland taste make possible the formulation of a number of food products with better consumer acceptability andgreater palatability than those made with traditional fibers.Being nondigestible, RS can be used in reduced-fatand sugar formulations. RS has properties similar to fiber andshows promising physiological benefits in humans, which mayresult in disease prevention.

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