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Resistant Starch in Food Products

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ABSTRACT

Resistant starch (RS) is the sum of starch and products of its degradation that are not digested in the small intestine of healthy individuals. Resistant starch has been categorized into the following types: 1) physically inaccessible or digestible resistant starch (e.g. starch present in partially ground cereals), 2) starch that occurs in its natural granular form (potato starch granules resistant to pasting, 3) retrograded starch (present in cooked-and-chilled potatoes) and 4) starches chemically or physically modified to be resistant to amylases. RS reduces the energy value and glycemic index of food products. Starches accessible to microorganisms present in the large intestine are of particular importance. The fatty acids formed due to fermentation processes favourably affect the metabolic processes by reducing cholesterol and triglycerides in human body. The formation of butyric acid in the final portion of the alimentary tract is particularly important in prevention of colon and colorectal cancer. RS content can be increased by adding it to the food products or by special preparation of the food products.

Keywords: glycemic index, starch degradation, starch modifications

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INTRODUCTION

Starch is stored as a reserve material in a variety of seed, tubers, fruit and rhizomes. It is the main constituent of cereal and potato products, being energy sources for people all over the world. A relatively simple chemical structure of this natural polymer and its susceptibility to acid and enzyme hydrolysis may suggest that it is completely digested in the alimentary tract of humans. In general, starch present in products destined for human consumption, is easily digested by the human body. However, not all types of starch are completely digested. In the 1930s, Nowotny, a Polish researcher, discovered that a large portion of non-gelatinized starch was resistant to amylolysis (Nowotny 1938). In the 1980s his findings were also confirmed by a Japanese researcher (Sugimoto 1980). Englyst was the first who discovered a type of starch, referred to as resistant starch, which is not digested in the small intestine (Englyst *et al.* 1992), but has healthful effects on the colon as an important source of carbon for micrororganisms naturally existing in the colon. Fatty acids produced during fermentation affect metabolic processes, reducing the risk of colonal cancer (Annison and Topping 1994), cholesterol and triglyceride levels in blood (Hara *et al.* 1999). Resistant starch (RS) also reduces the calorie value of food products and the glycemic index (Leber-Metzger *et al.* 1996), which is of great importance in the diets for obese and diabetic people. However, susceptibility of starch to amylolytic degradation varies, and the reasons for these variations have not been yet fully explained. One of these reasons is a wide variety of botanical sources and the resultant differences affecting starch properties due to technological or culinary processing.

DIFFERENCES BETWEEN BOTANICAL SOURCES AND STARCH TYPES

The size, shape and structure of the starch granules vary substantially between botanical sources and variations in starch granules from different plants may be observed microscopically (Cortella and Pochettion 1994). Potato starch granules are the largest (from 10 to 100 μm) among the raw material destined for starch manufacturing plants. Large granules of potato starch are oval in shape and their surface is uneven (seashell-like). Small granules are round with more even structure. Corn starch granules are much smaller (5-20 μm), polyhedron in shape. Rice starch is round and contains fine granules < μm, which occur in clusters. Wheat starch consists mainly of large (22-36 μm) granules coexisting with a number of small (2-5 μm) granules. The most original shape is that of cassava starch granules, which in a microscopic picture look like a hen's egg with a cut-off top (**Fig. 1**).

The characteristics of starch are differentiated not only in sizes and shape depending on the biological sources. Chemically, starch is a polymer consisting of glucose residues. Its granules contain two fractions varying in structure and properties: amylose which is a linear long chain (rather poorly-branched) fraction with α-1,4-glycosidic linkages and amylopectin containing both 1,4-glycosidic bonds and α-1,6-glycosidic linkages that occur in the branch-points of

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Invited Mini-Review

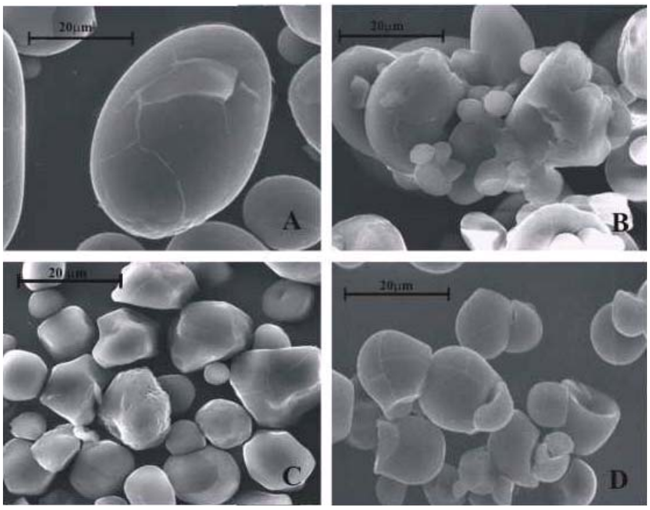

Fig. 1 Scanning electron microscopy of potato (**A**), wheat (**B**), corn (**C**) and tapioca (**D**) starches.

the short chain. The molecular weight (MW) of amylose is relatively low (<10^6 Da). The external chains of amylopectin form double helices, with the existence of growth rings of alternating crystalline and noncrystalline starch. Growing amylopectin molecules extend from the hilum (point of origin) to the surface of the starch granule. Six double bonds of these helices form semi-crystalline regions. On the other hand, amylose intertwined with amylopectin is present inside the starch granule and forms its amorphous regions. The crystalline and amorphous regions occur alternately and form a layered structure. The relative proportion and structural differences between amylose and amylopectin contribute to the significant differences in starch properties resulting from differentiation of the biological sources, molecular weight, sizes of starch granules, plant maturity and environmental conditions of plant growing (Leszczyński 2000). Native potato starch contains from 13 to 25% of amylose. There are also potato cultivars containing amylose-free, waxy starch. Amylose content of cereal starches also varies and may reach 70% (corn starch Hylon VII) (Singh *et al*. 2003).

Natural starch existing in form of granules contains not only carbohydrates, but also protein and lipid- compounds, which in wheat starch form protein-lipid net-like structures both on the surface and inside starch granules. Cereal starches contain about 0.5% of protein and 1.5% of fat. Some lipid portions penetrate the hydrophobic amylose helices thus forming amylose-lipid complexes. Potato starch contains only trace amounts of protein and fat.

Starch contains also from 0.2 to 0.6% mineral substances, mainly phosphorus present in cereal starches. It exists in combination with fat forming phospholipids or in esters of potato starch affecting its ion-exchange properties (Singh *et al*. 2003).

Water is also an important constituent of starch in which it occurs in free or crystalline forms. Starch contains about 20% of water in the air and this volume can be increased to 30% when starch is immersed in cold water. It is a completely reversible process when the temperature of water is < 40°C. The quantity of bound water depends on the crystalline form of starch. There are three types of starches, designated as type A, type B and type C, identified based on X-ray diffraction patterns. They depend partly on the chain lengths making up the amylopectin structure, the density of packing within the granules and the presence of water (Wu and Sarko 1978). Type A starch, which is common in cereals, exhibits high density of granules and low water content (4 water molecules per 12 glucose residues). Type B starch is typical of potato tubers, its structure is looser and contains more water (36 molecules per 12 glucose residues). Type C starch is typical of legumes (peas and beans) and is a combination of type A and type B (Leszczyński 2000).

The structure and composition of starch granules have an impact on starch properties. Depending on the biological sources, starches show variations in pasting temperatures (Singh *et al*. 2003), water absorption, and viscosity of pastes. Potato starch exhibits the greatest water holding capacity (WHC) of all the starches of industrial significance in contrast to cereal starches with low WHC and poor viscosity of pastes. The technological value of starch is frequently determined with regard to its pasting properties measured with the use of the Brabender Viscograph. Maximum viscosity of the pastes made from potato starch is several times as high as that of pastes made from cereal starches (Lewandowicz and Fornal 2008). Starch properties depend not only on the plant species, but also on the cultivar, maturity, soil environment, fertilizer use, irrigation, pesticide treatments and weather conditions (Leszczyński 2000).

Starch pastes, especially under refrigeration conditions, have a tendency for retrogradation, i.e. returning to insoluble aggregated or crystalline structures. This process causes turbidity of gels, decreases their viscosity and results in syneresis. Retrogradation is mainly due to amylose, which retrogrades faster than amylopectin. For this reason, researchers carry out studies aimed at obtaining plants low in amylose. However, these studies are expensive and time-consuming and besides, from social point of view, genetically modified plants are still unacceptable. Starches can be modified by simpler and less expensive methods.

STARCH MODIFICATIONS

Industrial applications of unprocessed starches are limited. They are mainly used as thickeners and ingredients of jellos and puddings. The properties of modified starches allow for a variety of functional applications. Starches can be modified by enzymatic and physical methods or combinations of both.

A major group of products obtained with modified starches comprises products of starch hydrolysis (maltodextrins, maltose, starch and glucose syrups). They can hardly be called modified starch products, especially those of high saccharification degree. In fact, these processes result in the production of new products. However, traditionally it has been accepted that each method of starch processing is a form of its modification. Starch hydrolysates are obtained by enzymatic methods or acid hydrolysis (Nebesny and Rosicka-Kaczmarek 2008). Maltodextrins are obtained from starch by α-amylase hydrolysis. They contain mainly high molecular weight sugars the properties of which depend on the dextrose equivalent (DE), i.e. a percentage of reducing sugars in conversion to glucose. The DE of maltodextrin is considered low when it is within the range from 7 to 10 and high when the DE ranges from 23 to 35. Maltodextrins are used for the production of nutritional food for children, confectionery and baked products as well as instant soups and gravies. Maltose syrups are obtained in starch reactions with α-amylase and β-amylase, resulting in the production of 60% of maltose (or 85% with the use of pullalanase). They are used in the production of candies, caramel, jams and ice-cream, giving the products a creamy, smooth texture. A wide array of starch syrups can be produced either by acid or enzymatic methods, depending on the DE (10-70) and the technologies used. Starch syrups are commonly used in candy and baking industries, breweries and fruit and vegetable processing technologies. Glucose is obtained from potato starch by enzymatic hydrolysis with amyloglucosidase after degrading it with the use of α-amylase. Crystalline glucose is used in candy manufacturing, vegetable processing, ice-cream and beverage production. It is also an important raw material for a pharmaceutical industry.

The most efficient methods used for starch modifications resulting in desired changes in starch properties are chemical methods. They do not affect the structure of starch granules, but result in slight changes in the chemical structure of the polymer. Almost all the glucose residues in starch chains have 3 free hydroxy groups participating in the reactions. They can undergo oxidation, etherification and esterification (Tomasik 2000). The properties of starch obtained in these processes differ from those of native starch and depend on the type and conditions of the reactions, substitution degrees and starch properties. The best raw materials for chemical modifications are potato and tapioca starches (Lewandowicz and Fornal 2008).

Oxygenized starch (E 1404) is obtained with sodium hypochlorite on starch suspension in alkaline solution. This reaction also leads to depolymerization. Oxygenated starch is soluble in cold water and forms transparent gels of low viscosity, slightly susceptible to retrogradation. Starch ethers are obtained from the reactions with monochloroacetic acid (carboxymethyl starch), ethylene oxide (hydroxymethyl starch) or propylene oxide (hydroxypropylene starch). These modifications increase pasting temperatures, solubility in water and viscosity of the pastes. The starches obtained in these modifications exhibit reduced susceptibility to retrogradation. Esterification is the most common chemical modification of starch. It is performed both with organic and inorganic acids and their derivatives as well as anhydrides (Golachowski 1998). The most common modified starches used in food industries are: acetylated starch (E 1420), distarch phosphate (E 1412), acetylated distarch phosphate (E 1414) and acetylated distarch adipate (E 1422). Acetylated starch is usually obtained by esterification in aqueous solution with acetic anhydride in alkaline environment. This type of modified starch exhibits lower pasting temperature and higher viscosity of the pastes as compared to native starch. Besides, it forms stable gels resistant to retrogradation. Distarch phosphate is a net-like starch, in which the modifying residue associates with two adjacent starch chains through the ester linkage. The cross-linked reaction of netting is performed in an aqueous solution at temperature ranges between 20 and 50°C in alkaline solution of phosphate oxychloride or sodium trimethaphosphate. Pastes made with distarch phosphate are highly resistant to mechanical forces and temperature changes. For this reason, this type of modified starch is used in meat products manufacturing, e.g. canned meat products subject to sterilization. Acetylated distarch phosphate and acetylated distarch adipate are obtained by double modification: netting and acetylation. Acetylated distarch phosphate forms transparent stable gels exhibiting high viscosity and resistance to temperature changes. Acetylated distarch adipate exhibits high stability in acid environment of the gels made from this type of modified starch (Golachowski 1998).

Modified starches with low degree of substitution have been accepted by law as food ingredients. Also starches of higher degrees of substitution are produced on an industrial scale, e.g. in textile and paper industries, and also for glue production, dehydrating substances, molding materials, drilling fluids or flocculants.

Starch properties can also be modified physically. Mechanical energy causes damage to starch granules and changes morphological and physical properties of starches. The longer the exposure to mechanical energy, the better solubility in cold water, water holding capacity (WHC) and susceptibility to amylolytic enzymes activity (Tester 1997; Leszczyński 2000). The viscosity of pastes made from damaged starch is reduced. Ion irradiation at doses >7 kGy can modify the structure of starch granules. Starch exposed to gamma irradiation increases in its capability, acidity and solubility and simultaneously the molar weight of the products decreases are observed (Duarto and Rupnow 1994; Gambuś *et al*. 1995). Prolonged heating of starch in water at 50°C reduces granule swelling, pasting temperature and amylose content (Larsson and Elasson 1991). On the other hand, heating of dehydrated starch at high temperatures may result in dextrinisation, increased solubility in water, reductivity and susceptibility to enzymes activity, even pyrolysis. Roasting of starch with acid used as a catalyst has found applications in dextrin production. Great changes in starch properties are induced by extrusion. It is a thermo-mechanical process, whereby the raw material is transformed into plastic mass in a short time, and next, due to rapidly decreasing pressure and water evaporation, a product exhibiting a characteristic texture and shape is developed. Extrusion changes crystallinity, structure and spatial ordering of starch granules, increases solubility in water and decreases viscosity of pastes. The higher the temperature of the process and the lower water content of the product prior to extrusion, the greater the changes (Chinnaswamy *et al*. 1989; Bindzus *et al*. 2002).

Commonly used starch modifications often affect its spatial structure, thereby inhibiting access of amylolytic enzymes to the starch chain, which consequently causes partial resistance of starch to amylolysis.

RESISTANT STARCH (RS) – DEFINITION AND TYPES

Resistant starch (RS) is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of a healthy man. It is subdivided into 4 fractions: (Haralampu 2000): RS 1 – is resistant because it is in a physically inaccessible form, indigestible by body enzymes (e.g. starch in partly milled cereal grain), RS 2 – of compact structure, non-pasting, insusceptible to various amylases (e.g. potato starch amylase), RS 3 – retrogradable starch (formed during cooling of cooked starch products) and RS 4 – chemically or physically modified starch.

RS1 and RS2 have little or no impact on human health. RS1 is the physically protected form of starch, found in whole grain, practically inaccessible for the microflora inhabiting the colon. RS2 loses its resistance during thermal treatment and culinary processing. Exceptional in this respect proved to be a high-amylose corn starch hybrid ae-VII which exhibited 30-40% resistance after tempering (Würsch 1999). RS3 and RS4 have a wider range of applications. RS3 results from retrogradation, which is the effect of gelatinized starch chains association and ordered structure. At lower temperatures, within a few hours, a major portion of amylose is folded into thermostable double helices ordered into crystalline structures over a particular region of the chain, interspersed with amorphous, enzyme degradable regions. Amylopectin, due to its branched structure, forms crystalline regions only outside its particles and therefore needs more time for retrogradation. The solubility temperatures depend on the botanical origin and retrogradation conditions. They range from 120 to 150°C for amylose and from 30 to 80°C for amylopectin (Silverio *et al*. 2000). Presumably, the resistance of retrograded starch depends on crystallinity and thermostability of precipitated starch (Leszczyński 2004). Amylose preparations containing RS3 are of particular interest due to their thermostability. In general, thermal operations do not affect starch resistance. The products containing RS, available on markets, are primarily obtained from high-amylose corn starch. The yields of thermostable crystalline forms can be increased by pullalanase or isoamylase treatments of starch pastes, which degrade branched α-1,6-glycosidic linkages in amylopectin (Würsch 1999), allowing linear chains (of appropriate length) to participate in the development of amylose retrograded structures (Guraya *et al*. 2001).

RS4 is modified chemically or physically and its resistance to amylase is due to the changes in the composition and the structure of molecules undergoing during modifications. Acetylation, phosphorylation, hydroxypropylation, netting with epichlorohydrin, saturation with iron ions or roasting with glycine increase starch resistance to enzymetic degradation (Sitohy Ramadan 2001; Hoover and Zhou 2003; Masłyk *et al*. 2003; Gryszkin *et al*. 2004; Le Thanh *et al*. 2007). Resistance of starch preparations increases with increasing substitution degree and parallely performed chemical modifications (Hoover and Zhou 2003). Reductions in starch digestibility can as well be due to its physicochemical interactions with some substances, e.g. lipids absorbed by amylose helices (Cui and Oates 1999; Tufvesson and Eliasson 2000) or fatty acids forming stable complexes with starch (Crowe *et al*. 2000). Tempering of starch at high temperatures and moisture content (Würsch 1999; Haralampu 2000) or extrusion processes (Ralet *et al*. 1990; Unlu and Faller 1998) are physical methods used for reducing starch susceptibility to amylases.

In chemical reactions, various substituents, which are linked with glucose residues, are introduced to the starch chains, and starch complex with lipids change the structure of starch helices, thereby reducing starch susceptibility to hydrolysis (Leszczyński 2004). "Tempering" of potato starch additionally extends double helices and inhibits crystalline defects (Genkina *et al.* 2003). Besides, in corn starch it results in the occurrence of new double helices of amylose (Teste *et al*. 2000). As a result, interactions between starch constituents occur consequently increasing the stability of starch granules. Due to these changes, starch solubility and its swelling capability are reduced and resistance of starch granules to amylolytic enzymes increases (Thompson 2000). Reduced susceptibility of extruded starch to amylases action is likely due to partial recrystallization (Leszczyński 2004) and repolymerization undergoing at high temperatures, which result in the occurrence of linkages that do not exist in native starch (Gryszkin *et al*. 2004).

HEALTH EFFECTS OF RESISTANT STARCH

According to the World Health Organisation (WHO) human populations both in developed and developing countries are threatened with increasing risk of such civilization diseases as: cardiovascular diseases, diabetes, cancer, and obesity. It is anticipated that the incidence of these diseases will increase from 46% in 2001 to 57% in 2020. In 2001 the disease contributed to 60% of human deaths all over the world (Anonymous 2003). One of the reasons for increasing the risk of these diseases is a poorly balanced high-energy diet, rich in highly-processed ingredients. Diet-dependent diseases are induced by insufficient supplies of fiber in food. Dietary fiber has a positive impact on physiological processes – it decreases cholesterol and glucose in human blood and stimulates bowel movements, thereby reducing the risk of gastrointestinal diseases, including cancer. Since the 1980s nutritionists have been carrying out studies on the effects of resistant starch in animals. It is well-known that the starch available to microorganisms inhabiting the colon has good effects, as it decreases the pH of the environment (Wronkowska *et al*. 2002). The increases in the pH of the digesta result from fermentation of resistant starch induced by the microflora and the formation of short-chain fatty acids (Soral-Śmietana and Wronkowska 2004), which stimulate the growth of various groups of beneficial bacteria in the colon, especially those of *Bifidobacterium* and *Lactobacillus* types, at the same time eliminating or inhibiting the development of pathogenic bacteria harmful to the body (Silvi *et al*. 1999). Since resistant starch is not digested in the colon and is able to restore the balance of bacteria in the digestive tract, it has been included in the list of prebiotics. Fatty acids produced in the colon improve metabolic processes, especially the synthesis of cholesterol and triglycerides (Hara *et al*. 1999), reducing their concentrations in blood. Butyric acid produced at the end of the digestive tract plays a key role in prevention of colon cancer. It is worth noting that the fermentation of resistant starch yields more butyric acid than the fermentation of non-starch polysaccharides (Annison and Topping 1994).

Food products containing RS, as compared to those containing digestible starch, are responsible for a slow release of glucose after eating, which consequently reduces the insulin response. It has been estimated that such effects are possible provided that a diet contains at least 14% of RS in relation to the total starch (Nugent 2005). The reducing glucose and insulin capability is especially precious for people with diabetes. Reducing the energy value of food, extended satiety due to slowly releasing glucose to the body and increasing gut-filling can find applications in the diets for obese people (Heijnen and Deurenberg 1995; Leber-Metzger *et al.* 1996). Besides, RS has a positive impact on bowel movements, which is very important in preventing constipation of obese people.

RS SOURCES AND INTAKE

RS accessible to the microflora of the large intestine (colon) is affected by internal and external factors. The internal factors depend on the structure of starch, such as its physiccal state, crystallinity and amylose content. The external factors depend on starch resistance to digestion in the colon. The latter include the amount of digested starch, amylose concentration in the digestive tract, the length of time needed for food transport in the digestive tract, the degree of starch conversion during processing and even chewing (Cierpikowska and Dryweń 1999).

Daily intake of RS is much lower in well-developed than in developing countries. This is mainly due to the amounts of processed food available on the markets and also eating habits. In Europe, a daily intake of RS *per capita* ranges from 2.5 to 8.5 g (Englyst and Hudson 1996; Brighenti *et al*. 1998; Nugent 2005) and as much as 40 g in developing countries. **Table 1** shows RS content of some starch-containing food products. The discrepancies in the results are likely due to the variations between the food products under investigation and in objective methods used for the analyses of RS content (Walter *et al.* 2005).

RS intake can be increased by food supplementation, i.e. by adding RS as an ingredient to breads and cakes as well as extruded food products (Yue and Waring 1998). Ready-made products high in RS2 which are available on the

Table 1 Comparison of RS parameters determined by 3 methods as described by different authors (Cierpikowska and Dryweń 1999).

Product	RS content (g/100 g of dry matter)		
	Biochemical method	Biological method *in vitro*	Biological method *in vivo*
Bread and bread rolls made from wheat flour	1	1.0	-
Whole-grain bread	1	2.6	-
Corn flakes	3	1.7	3.1
Oat flakes	2	0.4	
Boiled potatoes immediately after cooking	5	3.3	-
Boiled potatoes after cooling	10	-	-
Noodles immediately after cooking	5	2.6	-
Cooled noodles	4	-	-
Pearl barley (cooled after cooking)	9	5.1	5.5
Peas (frozen, cooked for 5 min)	5	9.6	-
Lentil (cooked for 20 min and cooled)	9	8.8	
Beans (cooked for 40 min)	18	25.5	-
Raw potato starch	75	-	-
High-amylose corn starch	17	-	-

From: **Cierpikowska M, Dryweń M** (1999) Skrobia oporna jako składnik żywności: wartość odżywcza i właściwości fizjologiczne. *Żywienie Człowieka i Metabolizm* **XXVI**, 147-155, ©1999 with kind permission from Instytut Żywności i Żywienia, Warszawa, PL.

markets today are: "Hi-Maize" made by the Starch Australia Ltd. (Australia), "Hylon VII" made by the National Starch and Chemical Company, "Amylomaize VII" made by the Cerestar Inc. and "Novelose 240" made by the National Starch and Chemical Company (the US). RS 3 is an ingredient of "Crysta Lean" made by the Opta Food Ingredients Inc. (the US) (Leszczyński 2004). The only product containing potato starch and manufactured on an industrial scale is "Pine fibre-C" made in Japan by the Matsutani Chemical Industry Co. Ltd. The latter is a soluble substance containing RS 4, obtained during physical (thermal) modification of starch, being a product of its thermal and enzymatic dextrinization (Wakabayashi *et al.* 1993).

The products containing modified RS can be used as functional food ingredients owing to their specific properties, which enhance health condition of humans and reduce the risk of chronic civilization diseases.

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