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RESISTANT STARCH – CLASSIFICATION, STRUCTURE, PRODUCTION

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Starch is a high-molecular carbohydrate composed of linear (amylose) and branched (amylopectin) chains of glucose residues. In water, at increased temperatures, it undergoes gelatinisation followed by amylase-induced hydrolysis. Owing to this, it is completely digested in the gastrointestinal tract of humans. Also raw starch of some plant species, *e.g.* cereals, is subject to complete but slow digestion. In addition, starch may occur in the form incapable of enzymatic hydrolysis, referred to as "resistant starch" (RS).

Resistant starch is a sum of starch and products of its degradation undigested in the small intestine of humans. There are four types of resistant starch.

Type I RS – physically unavailable starch. Amylolytic enzymes have no access to starch accumulated in undamaged plant cells as the gastrointestinal tract lacks enzymes capable of degrading the components of plant cell walls.

Type II RS – raw starch of some plant species, *e.g.* potato.

Type III RS – retrograded starch, *i.e.* spontaneously- or artificially-precipitated from starch paste, occurring in the form of water-insoluble semi-crystalline structures. As a result of retrogradation, more thermostable structures are formed by amylose rather than by amylopectin. The amount of resistant starch produced this way increases along with the increasing amylose content of starch.

Type IV RS – chemically- or physically-modified starch.

Resistant starch has been reported to reduce the caloric value of food products and to decrease glucose level in blood. In the large intestine, it is fermented by gut-colonising bacteria. Products of its fermentation include gases and short-chain fatty acids – acetic, propionic and butyric, which are responsible for favourable selection of intestinal microflora, reduce the levels of cholesterol, triglycerides, and urea in blood, as well as prevent the formation of gut cancer.

Commercial preparations of resistant starch are also produced to be used as food additives. Their resistance to the activity of enzymes, as well as physiological effects of their application, are determined with *in vitro* and *in vivo* methods. Results of those analyses are not always unequivocal, especially with the application of resistant starch with artificially-increased resistance. Resistant starch may serve as dietary fibre and exert a health-promoting effect on the human organism. Therefore further studies into its formation and results of its application in food production are still necessary.

STRUCTURE AND PHYSICAL PROPERTIES OF STARCH

Starch is a natural polymer occurring in all plant organisms. It is the major component of most of plant-originated foodstuffs and feedstuffs and of numerous industrial raw materials. Being synthesized from glucose, which is formed from dioxide and water, starch is an indirect product of photosynthesis, hence it is called renewable raw material.

Starch is a polysaccharide composed of the chains of glucose residues bound with a glycosidic linkage, however the structures formed are spatial in character. It contains two fractions: amylose and amylopectin. Amylose is a linear (rather poorly-branched) fraction with a medium degree of polymerization (reaching 10^2–10^3) [Murugesan *et al.*, 1993]. In amylose, glucose residues are bound with α-1,4-glycosidic linkages. Amylose chains with a high degree of polymerization contain from few to a dozen or so lateral branches. Glucose residues of those branches are bound to the main amylose chain with α-1,6-glycosidic linkages. Amylopectin, with a degree of polymerization reaching 10^6 [Aberle & Burchard, 1999], is a debranched fraction in which, apart from α-1,4-glycosidic bonds, also α-1,6-glycosidic linkages occur in the branch-points of the chains. Terminal branches of amylopectin, with the length of several to thirty glucose residues, form "clusters" (bunches).

In starch of some plant species, *e.g.* potato, amylopectin is partially esterified with orthophosphoric acid (V), however it binds mainly a hydroxylic group at C6 (*ca.* 61%) and C3 (*ca.* 38%) of glucose residue [Hizukuri, 1996]. One phosphate group occurs per 200 glucoside residues on average [Muhrbeck *et al.*, 1991]. Phosphate groups usually appear in amylopectin chains formed by 28–80 glucoside residues, where they are located no closer than the ninth glucose residue from the debranching site of the chain [Lisińska & Leszczyński, 1989]. Phosphate groups are linked with calcium, magnesium, sodium, and potassium cations by means of ionic bonds.

The proportion of amylose and amylopectin fractions depends on the botanical origin of starch. The amylose content of starch usually ranges from 10 to 35%, although in high-amylose starches it may reach even 70%, compared to the so-called "waxy" (high-amylopectin) starches where

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amylose occurs in trace amounts, *i.e.* from *ca.* 0% to *ca.* 4%. Under natural conditions, starch chains appear as left-handed spirals (helices) with six glucose residues per turn. The primary hydroxyl groups of glycoside residues are directed outwards the helix, whereas the secondary ones and hydrogen bonds are pointed inwards. The outer side of the helix is hydrophilic and its inner tunnel – hydrophobic in character. Owing to this, different water-insoluble substances, including lipid compounds or iodine, may penetrate into the starch helix, especially into amylose. The amylose-iodine complex is navy blue.

The amylose chains and arranged in parallel amylopectin branches (with a polymerisation degree of 10–30) form double helices with neighbouring chains which are strengthened with hydrogen bonds. Six neighbouring left-handed double helices make up crystalline forms, of *ca.* 10 nm in length [Eliasson & Gudmundsson, 1996]. At the sites where bunches (clusters) of double helices with an appropriate (over 10 glucoside residues) length appear, crystalline regions of starch are formed. In the areas where branches of amylopectin chains, its short chains or single helices of free amylose or amylose bound with lipid substances occur, either semi-crystalline or amorphous layers are likely to form. The crystalline layers formed from repeating subsequent amylopectin clusters together with amorphous substance present between crystals and crystalline layers form spherical structures ("blocklets"), 20–500 nm in size. The hard (crystalline) layers are composed of large "blocklets" (50–500 nm), whereas the soft (semi-crystalline) layers – of smaller ones (20–50 nm). Regions are arranged concentrically, the "hard" ones alternately with the "soft" ones [Kossman & Lloyd, 2000]. Starch crystallinity, positively correlated with the content of amylopectins and the number of double helices, ranges from 15% to 45% depending on plant species [Eliasson & Gudmundsson, 1996].

On the basis of X-ray diffraction spectrum, three crystallinity types of starch are distinguished: A, B, and C. Starch polymorphism results from a different length of lateral amylopectin chains and from the degree of order of double helices. In A-type starch, double helices of chains, usually 10–12 glucose residues in length, crystallizing in a hexagonal system, are densely packed, with a small share of crystallisation water (4 water particles per 12 glucose residues). The B-type crystals with a pseudo-hexagonal system are formed by rather loosely arranged double helices of chains, 13–18 glucose residues in length, with the share of a considerable number of water particles (36 per 12 glucose residues), grouped mainly in the centre of the crystal "cell". The C form is considered a mixture of A and B forms [Gernat *et al.*, 1990]. Type A crystallinity appears in starch of multiple cereals (wheat, maize, oat, rice) and of some root plants (tapioca, sweet potato, taro). Type B is typical of root and tuber-bearing plants (potato, jam) and some cereals (high-amylose: barley, maize, rice). Type C crystallinity has been observed, among other, in a number of leguminous plants. In starch of different maize species, containing from 0% to 84% of amylose, an inverse correlation has been observed between amylose content and a degree of crystallinity. Low-amylose starches form crystalline structures of chains with an average polymerization degree of 20 glucose residues, with short chains (10–13) predominating, and are characterised by a high degree of type A crystallinity. On the contrary, high-amylose starches with a low degree of crystallinity form type B crystals made of long chains with 35 glucose residues on average. Along with increasing starch hydratation (10–30%), its crystallinity is also observed to increase [Cheetham & Tao, 1998].

In plant tissues starch occurs in the form of structures composed of a high number of particles. Those structures, called granules, demonstrate a less or more regular, plain or complex, variety-specific shape. Their size (average) fluctuates, depending *i.a.* on the botanical origin, from 0.5 μm for amaranth to over 100 μm for canna. The regularity of starch chain ordering in a granule is reflected by its properties, namely the above-mentioned X-ray spectrum and the phenomenon of anisotrophy. The latter consists in the appearance of luminous granule sections in the polarised light in the microscopic image, taking the shape of the Maltese Cross.

In the light passing under the microscope, spherical lamination – the so-called "growth layer" – can be observed on starch granules. It results from different refraction of light in alternating crystalline and amorphous layers. The granule surface is characterised by the occurrence of numerous irregularities and pores of a different diameter and inside-granule depth [Juszczak *et al.*, 2003a, 2003 b]. The granule surface features are determined by the botanical origin of starch and, along with an increasing size of granules, they affect the specific surface area of starch. The specific surface area is diversified depending on the type of starch and ranges from *e.g.* 0.243 m^2/g in the case of potato starch granules with type B crystallinity to 0.687 m^2/g in the case of type A maize starch granules [Fortuna *et al.*, 2000]. The specific surface area of starch granules and pore volume are correlated with gelatinisation temperature and the viscosity of pastes obtained [Fortuna *et al.*, 2000]. The specific surface area of starch granules, as well as the number and size of pores, are also linked with the ability of starch to adsorb different substances, including protein compounds and enzymes, and with its susceptibility to the effects of multiple external factors.

Starch granules, apart from carbohydrate substance and water whose content depends on *i.a.* relative humidity of the ambient atmosphere, contain some amounts of lipid-, nitrogen-, and mineral compounds. Their contents are determined by the species of the plant the starch originates from. In potato starch, *e.g.* lipid- and protein compounds occur in trace amounts, whereas in cereal starches – the content of proteins exceeds 0.5% (in starches of grain legumes even 0.9%), and that of lipid compounds is higher than 1.5% [Eliasson & Gudmundsson, 1996]. Those substances form protein-lipid net-like structures overgrowing the granule's interior and occurring on its surface. Some lipids are bound to phosphorus, thus forming phospholipids. A part of lipid substances binds with amylose, thus penetrating into the hydrophobic interior of its helices. It affects the functional properties of starch, including viscosity of starch pastes, their transparency, *etc*. Starch contains also from 0.2% to 0.6% of mineral compounds, mainly phosphorus present in phospholipids or esters bound to metal cations.

Starch granules are not soluble in cold water; at increased temperatures (over *ca.* 40°C) and excess of water, they are subject to gelatinisation forming aqueous colloidal

solution, called starch paste. An initial symptom of the gelatinisation process is the disappearance of both anisotropy of the granules and starch crystallinity. Gelatinisation results in hydratation (especially of amorphous regions) and swelling of the granules to the volume higher by a few dozen, or even a few hundred than the initial one. With successively increasing temperatures (to 80–95°C), the next stage of gelatinisation involves partial depolymerization and water solubilization of carbohydrate substance. At the initial stages of gelatinisation of most starches, amylose and low-polymerized chains of amylopectin are transferred into the solution [Eliasson & Gudmundsson, 1996]. The resultant paste contains dissolved carbohydrate substance and fragments of starch granules. The gelatinisation temperature of the granules and the rheological properties of pastes obtained depend on the botanical origin of starch and different factors affecting it.

After cooling, starch paste gelates and forms compact jelly in which the starch substance binds considerable amounts of water. When stored for a longer period of time, especially at a temperature approximating 0°C, paste or starch gel is subject to changes referred to as aging. The viscosity of pastes reduces and some amount of water appears on the gel surface, indicating the phenomenon of syneresis. These changes proceed as a result of starch retrogradation which consists in the transfer of some amount of solubilized starch into an insoluble form. It results in lower starch concentration in the paste, thus causing a decrease in its viscosity. The phenomenon of syneresis observed in starch gel points to a decreased content of solubilised starch (binding water) in the gel.

The retrogradation process consists in gradual winding up of straight chains of starch solubilised in water (especially amylose) into helices, which in turn bind into double helices strengthened with hydrogen bonds, and in simultaneous dehydration. That process proceeds to a higher extent in starch pastes stored at lower temperatures. Vicinal double helices form permanent, thermostable and water-insoluble crystalline structures. When long amylose chains wind up, they may participate in the formation of multiple structures of this type. The structures obtained represent the B type of crystalline pattern, and the regions filled with non-crystallized fragments of amylose chains demonstrate amorphous character. In consequence, the retrograded starch is of a semi-crystalline character [Hoover, 2001]. A degree of starch retrogradation is observed to increase along with an increasing amylose content. Amylopectin is also subject to retrogradation, however due to its branching and shorter chains (*ca.* 15 glucose residues on average) which form double helices, the resultant crystalline structures demonstrate lower thermostability. It is worth emphasizing that in order to undergo rehydration the products of amylose retrogradation must be heated at temperatures over 120°C, whereas those of amylopectin retrogradation require temperatures slightly over 60°C [Botham *et al.*, 1994].

Gelatinised starch easily undergoes hydrolysis with amylolytic enzymes. In the initial phase of hydrolysis, under the influence of those enzymes (especially α-amylase), it is subject to partial depolymerization, which in turn is accompanied by the formation of dextrins, namely chains of glucoside residues with different degrees of polymerization. The final stage of hydrolysis, especially upon the activity of glucoamylase, results in the production of glucose. This way starch is digested in the human body. The processes described have also been implemented into industrial practice.

TYPES AND STRUCTURE OF RESISTANT STARCH

Concept and classification of resistant starch

As a carbohydrate occurring in food products, especially cereal- and potato-based ones, and in organs of some tropical plants, starch provides the organism with necessary energy approximating 4 kcal (16.7 kJ) per g. As a result of the activity of amylolytic enzymes of the gastrointestinal tract (also *in vitro*) starch undergoes hydrolysis. Hence it is regarded a compound rapidly and completely digested and absorbed in the small intestine in the form of glucose being a product of hydrolysis. It has been shown, however, that it only refers to starch subjected to thermal treatment in an appropriate amount of water (*i.e.* in a gelatinised form) and consumed immediately after being prepared. Also raw starch of some plant species (*e.g.* cereals), occurring as non-gelatinised granules, has been demonstrated to undergo complete, although slow, digestion. Therefore starch has been divided into: rapidly digestible starch (RDS) – degraded to glucose within 20 min upon enzymatic activity, and slowly digestible starch (SDS) degraded to glucose with the successive 100 min [Englyst *et al.*, 1992].

Some part of consumed starch has been observed to be incompletely digested and in the intact form or as products of its partial hydrolysis to escape the small intestine and enter the large bowel. This part of starch has been termed "resistant starch" (RS). It constitutes the difference between the amount of starch subjected to the activity of a complex of amylolytic enzymes and the amount of glucose (as starch equivalent) produced as a result of hydrolysis with those enzymes. By definition, resistant starch is the sum of starch and products of its degradation not absorbed in the small intestine of a healthy human.

Resistant starch has been found to appear in four forms. The early classification involved only three forms of starch [Englyst & Cummings, 1987; Englyst *et al.*, 1992]: **RS 1** – physically inaccessible starch; **RS 2** – starch of raw (non-gelatinised) granules of some plant species; **RS 3** – retrograded starch.

In successive years, those forms were supplemented with another one [Eerlingen & Delcour, 1995; Brown, 1996; Haralampu, 2000], namely – **RS 4** – chemically- or physically-modified starch.

Resistant starch I

Type 1 resistant starch (RS 1) includes starch present in cells of plants with undamaged cell walls, occurring *e.g.* in not completely ground cereal grain. It is unavailable to amylolytic enzymes, since the gastrointestinal tract lacks enzymes capable of degrading cellulose, hemicelluloses, lignins, and other constituents of plant cell walls. Therefore, such starch together with fragments of plant tissue passes the small intestine in the intact form.

Resistant starch II

Type 2 resistant starch (RS 2) includes granules of raw starch of some plant species, *e.g.* potato or banana. The

resistance of raw potato starch to the activity of amylolytic enzymes was first observed in 1937 by a Polish scientist – Nowotny [Nowotny, 1938]. By subjecting the raw starch of numerous plant species to the activity of an amylolytic preparation, Nowotny has observed the potato starch to undergo enzymatic hydrolysis to a small extent. Forty years later, similar results were reported by Japanese researchers [Fuwa *et al.*, 1977; Sugimoto, 1980]. Further investigations have also confirmed their results [Kelly *et al.*, 1995].

The phenomenon of raw starch resistance to the activity of amylolytic enzymes has not been fully explored yet. The resistance of raw potato starch has been attributed to large sizes of its granules, hence the limited area of their availability to enzymes [Ring *et al.*, 1988]. Still, the fine-grain high-amylose maize starch demonstrates the same resistance to enzymatic activity as the coarse-grain potato starch does [Planchet *et al.*, 1995]. Starch hydrolysis requires enzyme adsorption on the surface of starch granules [Leloup *et al.*, 1992]. Its extent is determined by the size and structure of starch granule surface. The specific surface area of potato starch granules is few times smaller than that of cereal starches. It seems, therefore, that a considerably lower degree of enzyme adsorption on the surface of potato starch granules than on the granule surface of some cereal starches may be the reason for a difference in the susceptibility of those starches to the activity of amylases. No relationship has, however, been reported between the extent of enzyme adsorption on granules of different starches and the degree of their hydrolysis [Kimura & Robyt, 1995].

Potato starch contains relatively high amounts of amylopectin and demonstrates a relatively high degree of crystallisation. The amylolytic enzymes first degrade the amorphous regions, hence crystallinity of starch granules could have been the reason for their resistance to the activity of those enzymes. Still, the degree of starch crystallinity is not always linked with its resistance to the activity of amylases. Cereal starches, characterised by a high crystallinity degree (type A), are susceptible to the enzymatic activity, compared to potato starch (type B), demonstrating a twofold lower crystallinity degree, which is resistant to enzymatic hydrolysis [Quingley *et al.*, 1998]. An increasing amylose content of maize starch is accompanied by a decrease in its crystallinity degree and increased resistance to enzymatic hydrolysis. Waxy maize starch, containing nearly 100% of amylopectin, demonstrates 40% crystallinity and is susceptible to the activity of amylases. The crystallinity of high-amylose enzyme-resistant maize starch accounts for 15% [Brown, 1996]. That relationship does not, however, pertain to all types of high-amylose maize [Fujita *et al.*, 1989].

Potato starch, similarly to high-amylose maize starch resistant to the activity of amylolytic enzymes, crystallises to the B form. Also starches of grain legumes, demonstrating the crystalline pattern of C type being a mixture of forms A and B, are to a high extent resistant to enzymatic hydrolysis [Garcia-Alonso *et al.*, 1998]. Starch granules of different plant species subjected to the activity of amylases have been observed with the use of an electron microscope [Fuwa *et al.*, 1977; Sugimoto, 1980; Soral-Śmietana, 2000]. Those observations indicated that the granules of cereal starch (type A) undergo deep enzymatic hydrolysis. They are centrally degraded by α-amylase, which results in the formation of tunnels directed towards granule interior accompanied by solubilisation of amorphous layers on the granule rim. In the case of potato starch granules (type B), the enzymes cause damage only to the surface of the granules, and their activity results in the formation of small pits [Sugimoto, 1980; Sarikaya *et al.*, 2000]. It may indicate that the B type starches, whose shape is formed by large "blocklets", are more resistant to the activity of amylases, compared to the A type starches.

The resistance of starch granules to the activity of amylases may be enhanced by annealing of starch which consists in keeping the starch for a longer period of time in water with a temperature lower than that of gelatinisation. Granules kept in water at a temperature of 20°C increase their volume by *ca.* 30%. At higher temperatures, water diffusion into the granule interior is more intensive. Keeping starch in water at an increased temperature results in water penetration into the granule interior, hence in a slightly enlarged granule size. Hydrogen bonds are disrupted and water particles bind to released hydroxyl groups. At temperatures lower than the starch gelatinisation temperature, it does not bring damage to the granules, but changes their properties. The resultant changes are determined by the botanical origin of starch, temperature and time of annealing, as well as by the concentration of starch suspension in water. The annealing of starches of different plant species results in an increase in the crystallinity degree, and strengthening of crystalline forms of the granules, "stiffening" and ordering of starch chains both in the crystalline and amorphous layer. Those changes in the starch granule structure evoke an increase in temperature of starch gelatinisation and increased enthalpy of that process [Leszczyński, 1992]. Annealing of potato starch induces additional elongation of double helices and reduction of damage caused to crystals [Genkina *et al.*, 2003]. In maize starch, it is also responsible for the formation of new double helices with the share of amylose [Tester *et al.*, 2000]. Starch components are observed to interact and granule stability is increasing [Hoover & Vasanthan, 1994]. Those changes result in decreased solubility and swelling capability of starch, and in increased granule resistance to the activity of amylolytic enzymes [Hoover & Vasanthan, 1994, Thompson, 2000].

A similar effect can be obtained upon heating the granules of high-amylose maize starch at a temperature of *ca.* 100°C and moisture content below 35% [Ito *et al.*, 1999]. A decrease in a swelling degree and an increased resistance to enzymatic activity obtained in this "heat-moisture" process are likely to result from the formation of additional crystallites with the share of amylose, as well as from ordering and binding its chains in the amorphous regions [Hoover & Manuel, 1996]. In the case of potato starch, however, this process results in disorders of granule structure, change of the crystallinity pattern from B- to A- or C--type, and increased susceptibility to enzymatic degradation [Kuge & Kitamura, 1985]. Crystalline structures are degraded, a degree of starch crystallinity decreases, and double helices are disrupted in the amorphous regions. This, in turn, results in a decreasing degree of granule swelling, increasing temperature of their gelatinisation, and enhanced susceptibility to the activity of enzymes [Gunaratne & Hoover, 2002].

On the basis of the data presented above, it may be concluded that the resistance of raw starch of some plant species to the activity of amylolytic enzymes is mainly determined by the structure of starch granules, especially its B-type crystallinity. The granules of potato starch and high-amylose maize starch, resistant to enzymatic activity, demonstrate the B-type of crystallinity. Upon long-standing amylase activity, the content of amylose and the number of short chains of amylopectin are found to decrease and the gelatinisation temperature is observed to increase in those starches [Jiang & Liu, 2002].

On the surface of potato starch, there appear apparent layers resulting from alternate packing of spherically-oriented crystalline and amorphous regions. In a microscopic picture, those "growth layers" are considerably better visible on potato starch granules than on these of starches of other origin. The B-type crystalline structures of potato starch granules are made of double helices and form large "blocklets" incorporated in the "hard" crystalline layers of the granules. The crystalline layers of the outer part of potato starch granules are characterized by the presence of very large "blocklets", whose sizes range from 200 to 500 nm, whereas in the cereal starch granules, susceptible to enzymatic activity, the "blocklets" are remarkably smaller. When analysed with Atomic Force Microscopy (AFM) on a folded structure of potato starch granules, those large "blocklets" appear as convexities [Gallant *et al.*, 1997]. This technique enables also their observation on preparations from granule interior [Ridout *et al.*, 2002].

Considerable length of lateral amylopectins chains which form crystals and the occurrence of large "blocklets" may affect starch resistance to the activity of enzymes [Kossman & Lloyd 2000]. Their effect may be, however, differentiated depending on the plant species; *e.g.* granules of starch of one pea variety containing large "blocklets" demonstrate lower resistance than those of other pea variety with small "blocklets" [Gallant *et al.*, 1997]. It is worth emphasizing that also amorphous regions of enzyme-resistant granules are highly ordered. It may indicate that starch resistance is determined not only by amylopectin in a crystalline form. A number of starches with a higher content of amylose are more resistant to the activity of enzymes, compared to these with low amylose concentration [Gallant *et al.*, 1997]. Even within one species (papilionaceous plants), high-amylose small starch granules are more resistant to amylase activity than the large granules with low amylose content [Hoover & Zhou, 2003].

It should be assumed that starch resistance is also affected by other elements of granule structure, including the shape of its surface, size of pores, *etc*. For example, starch susceptibility to gelatinisation, resulting from these properties, may also be a factor influencing starch resistance to the activity of amylases. Granules of enzyme-resistant high-amylose maize starch are not completely gelatinised even at the boiling point of water [Brown, 1996].

Resistant starch III

Type 3 resistant starch (RS 3) is a substance precipitated from paste or starch gel in the retrogradation process. During starch gelatinisation, starchy substance partly depolymerized as a result of temperature-water interaction passes from swollen granules into solution, thus forming a colloidal water solution, called starch paste. At an appropriate concentration (over 1.5% of amylose or over 10% of amylopectin) and lowered temperature, the starch paste undergoes gelatinisation which proceeds in two stages. At the first stage, phases are subject to separation, as a result of which the solid phase of a polymer forms a net-like structure which binds the liquid phase in its meshes. At the second stage, double helices of amylose are formed in the polymer phase. The amylose gel occurs as a microporous structure made of threads, 10–30 nm in diameter. They are composed of joint segments of amylose chains, with a degree of polymerization ranging from 26 to 73, appearing in the form of double helices. Within a few hours of gel storage, the helices aggregate and form highly thermostable (dissolution temperature above 150°C) B-type crystalline structures. Apart from those structures, gel structure includes also amorphous phase made of loose amylose chains with the polymerization degree of 6–30 [Leloup *et al.*, 1992a]. During gel treatment with amylolytic enzymes, the amorphous fraction undergoes hydrolysis and further ordering of amylose chains proceeds, contrary to the crystalline fraction built of those chains, which remains resistant to the enzymatic activity [Colquhoun *et al.*, 1995]. The formation of resistant starch III is affected by lipid substances which occur in starch of numerous plants and form inclusive complexes with amylose, penetrating into its chains. Such amylose does not bind into double helices which form crystalline structures upon aggregation. Therefore, a lower number of insoluble crystallites of amylose are precipitated in the retrogradation process, thus less resistant starch is produced [Eerlingen *et al.*, 1994].

Amylopectin gels are also partly crystalline. Short outer branch-points of amylopectin with a polymerization degree of 14–20 link into crystalline structures, forming a net. Unlike amylose, the crystallisation of amylopectin proceeds very slowly. The amylopectin crystalline structures are less stable than those of amylose, which results from a limited length of their chains. Their dissolution temperature ranges from 55 to 70°C [Eerlingen & Delcour, 1995].

The crystalline structures formed in starch pastes and gels, growing during storage – especially at lower temperatures, reveal resistance to the activity of amylolytic enzymes. Retrograded starch, precipitated from pastes or gels, demonstrates a semi-crystalline character and only its part undergoes enzymatic hydrolysis. Its major part behaves typically of starch resistant to the activity of amylolytic enzymes [Shin *et al.*, 2003].

Storage temperature of starch paste affects the amount and character of resistant starch formed. When paste is stored for several hours at a low temperature, more resistant starch is formed than upon paste storage at high temperatures. Still, long storage of paste at a temperature approximating 100°C results in the formation of higher amounts of resistant starch than within the same time, but at lower temperatures [Eerlingen *et al.*, 1993]. Resistant starch formed at low temperatures demonstrates the B-type of crystallinity, whereas that produced during starch paste storage at the boiling temperature – the A-pattern of crystallinity [Shamai *et al.*, 2003]. At low temperatures, major part of amylose is subject to retrogradation and precipitation from the solution, at higher temperatures those processes proceed only in its fractions with a low degree of

polymerization [Lu *et al.*, 1997]. The length of amylose chains undergoing retrogradation has no impact on the polymerization degree of chains that form crystalline aggregates of resistant starch upon retrogradation [Eerlingen *et al.*, 1993a]. The size of the crystals formed is likely to depend on the botanical origin of starch and methods applied for their production. In amylose of wheat starch, their formation results from aggregation of chains with a degree of polymerization (DP_n) ranging from 19 to 26 [Eerlingen *et al.*, 1993a], whereas in that of potato starch – from aggregation of chains with DP_n of 39–52 [Lu *et al.*, 1997]. Retrogradation of pea starch amylose results in the formation of three main linear fractions, acknowledged as semi-crystalline products, with DPn exceeding 100, approximating 35, and below 5 [Cairns *et al.*, 1996]. The products of amylose retrogradation with both *in vitro* [Cairns *et al.*, 1995] and *in vivo* methods [Abia *et al.*, 1996], display properties of starch resistant to the activity of amylolytic enzymes.

Amylopectin is also capable of retrogradation, however in its case retrogradation is a long-term process requiring several to a dozen or so days of starch paste storage at an appropriate temperature. The process is more efficient when the starch paste is cooled and heated several times. Retrogradation of amylopectin results in the formation of crystalline structures built of chains with a polymerization degree ranging from 6 to over 50. Temperature of their dissolution ranges from 30° to 80°C, depending on the botanical origin of starch and conditions of the retrogradation process [Silverio *et al.*, 2000]. Amylopectin retrograding at low temperatures crystallizes into B-type structures even when crystallisation of raw starch granules corresponded to the A-type crystallnity. The products of amylopectin retrogradation are resistant to the activity of amylolytic enzymes [Eerlingen *et al.*, 1994a].

The resistance of starch retrogradation products to the activity of amylolytic enzymes is likely to result from their occurrence in the form of B-type crystalline structures. The same pattern of crystallinity is observed in amylase-resistant raw granules of potato starch or high-amylose maize starch. Higher resistance is displayed by retrograded amylose than by the products of amylopectin retrogradation. It results from considerably higher thermostability of the crystalline structures of retrograded amylose, compared to those formed upon retrogradation of amylopectin.

Resistant starch IV

Type 4 resistant starch (RS 4) includes starch modified chemically or physically (mainly by thermal treatment), or with both those treatments. Acetylated starch of papilionaceous plants is characterized by a relatively high degree of resistance to the activity of amylolytic enzymes. Similar properties are displayed by starch of papilionaceous plants modified by hydroxypropylation. Resistance of the above-mentioned starch preparations increases with an increasing degree of substitution [Hoover & Zhou, 2003]. Hydroxypropyl distarch phosphate exhibits twofold lower susceptibility to the activity of amylases compared to native starch. Some resistance to enzymatic activity is also demonstrated by acetylated distarch phosphate [Östergård *et al.*, 1988]. Resistance of starch increases with an increasing number of its chemical modifications applied simultaneously [Wolf *et al.*, 1999]. The properties of resistant starch are also observed in monostarch phosphate, however in this case the resistance degree increases along with a degree of substitution with phosphoric acid (V) [Sitohy & Ramadan, 2001]. A product of monostarch phosphate heating with glycine is characterised by substantially higher resistance to the activity of amylolytic enzymes than the monostarch phosphate itself [Masłyk *et al.*, 2003]. Heating of soluble starch saturated with iron (III) ions also decreases its susceptibility to the enzymatic activity [Leszczyński *et al.*, 2003]. Treatment of soluble starch or that with the addition of glycine with high temperatures inhibits, to a high extent, enzymatic hydrolysis [Kroh & Schumaher, 1996].

During heating of starch at high temperatures with or without the addition of acid acting as a catalyst, starch undergoes dextrinisation. Degree of starch depolymerization proceeding during this treatment and properties of dextrins formed depend on the botanical origin of starch and dextrinization conditions, especially acidity and temperature. Dextrins obtained under specified conditions demonstrate the properties of resistant starch [Ohkuma *et al.*, 1990]. The resistance of the resultant dextrins to the activity of amylolytic enzymes increases with a proceeding degree of dextrinization and elongated time of the process [Wang *et al.*, 2001].

The resistance of chemically-modified starches to the activity of amylolytic enzymes results from changes in the composition and structure of starch particle proceeding upon modification. As a result of chemical modification, different substituents are incorporated into starch chains and bind to glucose residues. Their presence and the resulting spatial changes in the chain are likely to hinder the arrangement of the enzyme next to starch, enabling its normal activity. The resistance of products of starch thermal depolymerization – dextrins – to enzymatic activity results from changes in their structure, compared to starch. Upon heating of starch, depolymerization, transglucosidation and repolymerization proceed in the interior of its particles. With elongation of the dextrinization process, an increase is observed in the number of 1,3 and 1,2 linkages between glucoside residues of resultant dextrins [Ohkuma *et al.*, 1990]. The free glucose formed adheres randomly to the chain, which results in the formation of different linkages between glucoside residues in dextrin, including these that do not occur in normal starch. These linkages cannot be disrupted by amylolytic enzymes occurring in the gastrointestinal tract of humans. Only glucoamylase has been claimed to be capable of disrupting α-1,3-glycoside linkages.

Reduced digestibility of starch may also result from its interactions with some substances, including *i.a.* lipid substances penetrating into the interior of amylose helices. Complexes of sago starch with monoglycerides of fatty acids in starch paste demonstrate reduced susceptibility to the activity of amylases [Cui & Oates, 1999]. The same phenomenon is also observed in other compounds, *e.g.* some fatty acids. Together with amylose chains, they form durable complexes which do not undergo hydrolysis with amylases. Such complexes are also formed at a temperature of 37°C. They are also likely to form in the small intestine of humans where fatty acids, released from lipids under the influence of lipase, may complex with the products of partial starch hydrolysis, thus increasing the amount of not-digested resistant starch passing to the large bowel [Crowe *et al.*, 2000].

Significance of resistant starch

Until recently, consumption of carbohydrate-rich foods was highly recommended, *e.g.* potatoes directly after thermal treatment inducing gelatinisation of potato starch, as it was known that starch retrogradation proceeds in such products, especially those cold-stored due to sanitary considerations. The retrogradation process diminishes the amount of starch digested, hence it lowers the nutritive (caloric) value of a product. Nowadays, economic conditions and viewpoints on nutrition have changed. It is expressed by a tendency to reducing the caloric value of meals, observed especially in developed countries. Civilizational changes have also contributed to an increased intake of dietary fibre, indispensable for proper functioning of the organism. It was the reason of an increased interest in resistant starch – a natural food component, believed to be neutral to the organism [Annison & Toping, 1994], increasing food weight, but not its caloric value [Ranhotra *et al.*, 1996].

The consumption of products in which a part of starch occurs as non-digestible in the small intestine decreases blood levels of both glucose and insulin (necessary for glucose metabolism), compared to products with the same amount of completely digestible starch [Bornet *et al.*, 1989]. Non-digested resistant starch passes through the small intestine, but in faeces occurs in small amounts only. It results from the fact that it is utilized by the intestinal microflora of the large bowel [Ebihara, 1992]. Similarly to dietary fibre, upon the activity of large bowel microflora, resistant starch undergoes fermentation which results in the formation of methane, hydrogen and short-chain fatty acids (mainly acetic, propionic, and butyric ones) [Robertson *et al.*, 2000]. Those acids decrease pH and affect the selection of microflora colonising the large intestine, thus stimulating the development of beneficial groups of bacteria and eliminating pathogenic microorganisms. The acids formed in the large intestine influence the organism's metabolism, *i.a.* decrease blood levels of triglycerides, LDL cholesterol, and urea. Butyric acid formed upon RS fermentation in the amounts higher than those formed as a result of non-starch polysaccharide fermentation, plays a key role in the prevention of colorectal and anal carcinoma The data presented indicate that starch is not only neutral to organisms, but also demonstrates health-promoting activity [Leszczyński, 2000].

Occurrence of resistant starch in food

Starch, even as resistant as that of native potato, subjected to thermal treatment in excess of water gelatinizes, thus becoming available to enzymes, and undergoes hydrolysis. It is, therefore, completely digested and assimilated by the organism. Still, different technological treatments and further processing of carbohydrate products are likely to induce the formation of some amounts of resistant starch in those products.

In different food products, a part of their starch is resistant to the activity of amylolytic enzymes. It depends on the botanical origin of raw material and changes proceeding under conditions of the production process. The content of resistant starch in food fluctuates from a fraction up to 20% of product's weight and is, to a high extent, determined by food preparation manner [Brighenti *et al.*, 1998]. During the production of bakery products and confectionery, the content of resistant starch is observed to increase [Marlett & Longacre, 1996]. A further increase in RS content in bakery products proceeds during their storage, mainly as a result of amylopectin retrogradation [Eerlingen *et al.*, 1994b]. An increased RS content in bakery products may be achieved by a change in technological parameters of the baking process, and by the addition of high-amylose starch or lactic acid [Lijeberg *et al.*, 1996]. The content of resistant starch in different cereal-based products depends on the raw material used and technological process applied [Ranhotra *et al.*, 1999]. The amount of resistant starch in rice, prepared for consumption with different methods, is determined by the amylose concentration in starch [Sagum & Acrot, 2000]. In fried dry cereal products, resistant starch content depends on the botanical origin of starch used for their production. During frying, RS content increases, however the increase is higher in the internal than in the external part of the fried products. In those products, resistance of RS starch to enzymatic activity is positively correlated with amylose concentration in starch used for their preparation. An increase in the RS content in fried cereal products is accompanied by their increasing hardness [Pinthus *et al.*, 1998].

During thermal treatment of potato tubers, starch gelatinizes and is almost completely digested. Freshly-made potato dishes contain 1–2% of resistant starch. However, the content of enzyme-resistant starch is observed to increase substantially upon multiple cooling and heating of those products [Kingman & Englyst, 1994]. As a result of cooked potato storage at a temperature approximating 0°C more resistant starch is formed than at room temperature [Gormley & Walshe, 1999]. Fried potato products, especially French fries, contain more resistant starch than cooked potatoes. They are also characterized by a higher glycaemic index compared to cooked potatoes [Garcia-Alonso & Goñi, 2000]. The content of resistant starch in French fries increases along with their increasing thickness. The starch is packed non-uniformly, it occurs in smaller amounts in the outer layers of the French fries. Treatment of potato starch and potato amylose and amylopectin preparations according to procedures applied at the production of French fries indicates that amylose is responsible for the appearing resistance of starch. In this case, the resistance of potato amylopectin is negligible [Goñi *et al.*, 1997]. In a variety of products, including freeze-stored ones, the contents of resistant starch increase depending on the origin of raw material they are made of [Rosin *et al.*, 2000].

Production and application of resistant starch preparations

The content of resistant starch in food products may be increased by supplementing them with special starch preparations used as an additive to raw material in the technological process. Those starch preparations with high concentration of resistant starch are produced with multiple methods and demonstrate different types of resistant starch. Food products may be enriched in RS I. It refers most often to dry cereal products or some types of bakery products. They are made of (or supplemented with) whole or partially desintegrated cereal grain [Stephen 1994]. In such material, starch - present in the cells with walls undamaged in the technological process – is inaccessible to amylolytic enzymes.

As a result of technological processing, RS II present in a variety of products (*e.g.* raw potato starch) is subject to gelatinisation and loses its resistance to the activity of enzymes. The exception is high-amylose maize starch whose granules are only partly gelatinised at usual temperature of most of technological processes (*ca.* 100°C). An exceptionally high degree of resistance to amylolytic enzymes is displayed by starch of ae-VII hybrid of that maize [Haralampu, 2000]. It contains *ca.* 70% of amylose and 12%–18% of resistant starch [Thompson, 2000]. In this starch, RS concentration may be increased by thermal treatments, including annealing in water at a temperature lower than that of gelatinisation, heat-moisture or induction of swelling without causing damage to the granule structure. Such treatments evoke an increase in RS content up to 30%–40% [Würsch, 1999].

A variety of commercial preparations with a high content of type II resistant starch are offered on the market, they include *i.a.* fine-grained "Hi-Maize" by the Starch Australia Ltd. (Australia) produced from high-amylose maize starch [Brown, 1996], natural high-amylose maize starch "Hylon VII" by the National Starch and Chemical Company (USA) or thermostable "Amylomaize VII" by the Cerestar Inc. (USA) being also natural high-amylose maize starch. The market offers also a preparation with enhanced resistance to amylases called "Novelose 240" made by the National Starch and Chemical Company (USA). The preparation is obtained by thermal modification of the granules of high-amylose maize starch hybrid. The modification consists in heating starch with a moisture content of 10–80% at a temperature of 60–160°C [Haralampu, 2000].

The retrogradation process of gelatinised starch induces the formation of type III resistant starch. Retrogradation affects most of amylose which forms water-insoluble thermostable semi-crystalline structures. In order to facilitate solubilisation of starch it is treated with high temperatures (over 100°C) and then cooled to precipitate retrograded sediment [Schmiedl *et al.*, 2000]. Organic solvents are often used in order to precipitate starch from a solution. [Lewandowicz *et al.*, 1998]. The intensification of RSIII formation is accomplished through the activity of pullulanase or isoamylase undoing α-1,6-glycosidic linkages in amylopectin. Debranching of lateral amylopectin branches results in the formation of relatively short additional chains of the linear fraction of starch undergoing retrogradation. Those products can be concentrated through their precipitation with alcohol preceded by keeping them in a saline solution [Würsch, 2000]. Cooling and drying of pastes made of starch treated with pullulanase results in obtaining a product with high resistance to the activity of amylolytic enzymes [Guraya *et al.*, 2001]. The RSIII formation may also be evoked by subjecting a starch product to the extrusion process [Gebhardt *et al.*, 1998]. The market offers an RSIII preparation called "CrystaLean" by Opta Food Integredients Inc. (USA), produced by starch retrogradation of high-amylose maize starch ae-VII hybrid. After solubilisation of starch at a high temperature (*ca.* 150°C), lateral branches of amylopectin are debranched enzymatically and the hydrolysate obtained (demonstrating reductivity of maltodextrin) is then subjected to a few cooling-heating cycles [Haralampu, 2000]. Another RSIII preparation available on the market is "Novelose 330" by the National Starch and Chemical Company (USA).

Preparations of chemically-modified starch are applied as food additives, thickening or gelling agents, fat replacers *etc*. When subjected to hydrothermal treatment in the course of technological processing of food, those starches are completely digested [Galiński *et al.*, 2000]. The availability of chemically-modified starches is determined by their origin and by the degree of their substitution with chemical groups [Wolf *et al.*, 1999]. Starch subjected to chemical modification is characterized by reduced susceptibility to the activity of amylolytic enzymes, hence it is included into type IV resistant starch. It refers especially to some types of modification. Mono-starch phosphates demonstrate reduced susceptibility to amylases, however the effect of their hydrolysis decreases along with an increasing degree of starch substitution with the phosphate [Sitowy & Ramadan, 2001]. Similarly, the higher the resistance of di-starch phosphate to the activity of amylases, the higher the degree of its substitution [Woo & Seib, 2002]. A high content of starch resistant to the activity of amylolytic enzymes is displayed by hydroxypropyl di-starch phosphate [Östergård *et al.*, 1988]. Its resistance depends on the degree of substitution with a hydroxypropyl group [Kishida *et al.*, 2000]. Starch cross-linked with epichlorohydrin inhibits the activity of α-amylase [Somers *et al.*, 1991]. Still, this type of starch is not allowable as food additive.

Although resistance of chemically-modified starch to the activity of amylolytic enzymes has been confirmed, there are no commercial preparations of this type of starch. The only RSIV preparation available on the market is "Pine fibre-C" by the Matsutani Chemical Industry Company Ltd. (Japan). It is a soluble substance produced upon physical (thermal) modification of potato starch, being a product of its thermal and enzymatic dextrinization [Wakabayashi *et al.*, 1993]. It displays a low degree of susceptibility to the activity of amylolytic enzymes, reduces cholesterol and triglyceride levels in blood [Wakabayashi *et al.*, 1991], and lowers glucose level in blood [Ueda *et al.*, 1993].

Preparations of resistant starch, used as food additives to lower its caloric value, are more commonly applied. They reduce the availability of some saccharides in food, but have no negative impact on the organoleptic properties of food products. The addition of components with artificially-increased RS content during baking of bakery products does not deteriorate the quality of products obtained [Eerlingen *et al.*, 1994b]. It has also been found not to diminish the organoleptic properties of extruded products and confectionery [Yue & Waring, 1998].

Prospects for the application of resistant starch

An increased consumption of highly-processed products causes reduction of dietary fibre in food. In Germany, in the years 1880–1970, the intake of dietary fibre decreased *ca.* 2.5-fold, whereas in Poland in the period of 1950–1995 – by nearly 20% [Sekuła, 1997]. Therefore, the production and application of resistant starch in food become a necessity. As it is not digested by the organism, resistant starch reduces the caloric value of food products, lowers glucose blood level, and increases demand for insulin. Moreover, similarly to dietary fibre components, it is fermented in the large intestine, which results in the production of components favourable to the composition of intestinal microflora. Fermentation products affect also the metabolism of

organic lipid compounds. Hence, resistant starch is treated as a component of dietary fibre. According to one of the definitions, dietary fibre is a sum of polysaccharides and plant lignins resistant to the activity of digestive enzymes of humans [Delcour & Eerlingen, 1996]. Some authors, however, find it improper to consider resistant starch a dietary fibre component, since starch is not a component of the plant cell wall not affected by human digestive enzymes, as it follows from other definitions of dietary fibre [Englyst & Hudson, 1997].

Resistant starch should constitute a "prebiotic", namely a medium for "probiotics", *i.e.* a part of intestinal microflora which demonstrates positive effect of the human organism. Not all types of starch, however, serve this purpose [Wronkowska *et al.*, 2002]. The occurrence of higher amounts of starch in faeces points to incomplete decomposition of resistant starch in the large intestine, which indicates inappropriate structure of the preparation applied. Such cases occur *e.g.* at the application of high-amylose maize starch subjected to the heat-moisture treatment [Ito *et al.*, 1999]. A similar phenomenon is observed at the application of hydroxypropyl starch characterised by a great resistance to enzymatic activity due to its high degree of substitution (over 0.6) [Würsch, 1999]. The hydroxypropyl starches are also found to exhibit laxative properties [Ebihara *et al.*, 1998].

The effect of resistant starch on the organism depends not only on the degree of its resistance to enzymatic activity. Its impact is, to a high extent, determined by its composition and structure of the preparation used. Not all types of resistant starch are found beneficial to the cholesterol level in blood [Wisker, 2000]. The intensity of short-chain fatty acid formation as a result of fermentation in the large intestine is not determined by the starch resistance degree, but by its composition and properties [Bednar *et al.*, 2001]. Those facts indicate the need for further research into the production and properties of resistant starch on the one hand, and into its effect of the organism on the other.

The resistance of starch is evaluated with a variety of *in vitro* and *in vivo* methods. *in vitro* determination of resistant starch content of food products consists in enzymatic hydrolysis of all substances except not dissolved starch and dietary fiber components not undergoing decomposition. Not decomposed starch is then dissolved in hydroxide, hydrolyzed and determined quantitatively. The differences between particular methods concern the reaction, time and temperature of incubation, types of enzymes, procedure of resistant starch dissolution, precipitation and quantitative determination [McCleary & Monaghan, 2002]. Among the methods where resistant starch is determined by enzymatic action at a temperature of 37°C, the essential difference is incubation time. In some of these methods it is 16 h [Faisant *et al.*, 1995], like in the new method recommended by AOAC [McCleary & Monaghan, 2002]. In the method simulating natural conditions of the alimentary tract of a healthy human, the incubation time is 120 min [Englyst *et al.*, 1992]. It seems that if consumed starch is to be digested in the gastrointestinal tract of a human, the whole process will not take longer than two hours. Starch should not be expected to stay there as many as 16 h. Furthermore, the Englyst method allows to determine the amount of starch hydrolyzed by digestive enzymes quickly (during 20 min) and slowly (during the next 100 min). Such measurements are of primary importance due to the blood level of glucose and insulin demand dependent upon slow or quick starch hydrolysis..

The results on *in vitro* determination of starch resistance should be confirmed by its *in vivo* determination. Experiments on animals are easier to perform in practice [Ito *et al.*, 1999]. Experiments carried out on humans are more difficult for practical reasons, but their results may be much closer to the actual ones [Cummings *et al.*, 1996]. Such experiments provide the basis for estimating the value and technological suitability of resistant starch preparations.

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FINAL REPORT

Title of the research ordered project:
THE METHODOLOGICAL BASES OF THE EVALUATION OF THE QUALITY AND SAFETY OF THE NEW GENERATION FOOD (PBZ-KBN-020/P06/1999).

Title of the individual project:
Physiological effect of selected resistant starches at different diets.

Institution:
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Leader:
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Key words
Potato starch, soluble starch, dextrin, chemically modification, resistant starch.

SYNTHESIS OF RESULTS

As a result of chemical modifications and then subjecting the products obtained to the activity of a microwave field or air-stream heating, a considerable number (*ca.* 100) of starch preparations were produced. The products of potato starch modification were characterized by relatively low solubility, and substances obtained upon the modification of soluble starch, white dextrin and maltodextrin demonstrated high solubility in water. Both chemical and additional physical modifications induced substantial changes in properties of the preparations examined. In this investigation, their susceptibility to the activity of amylolytic enzymes was found their most important property. The other characteristics analyzed were linked to that property of experimental preparations. Of all preparations produced, three were selected for biological analyses. Their selection was based on their resistance the activity of amylolytic enzymes (approximating 50% compared to the non-modified material) and relatively uncomplicated production process. Diversity of their solubility in water and water absorption were also taken into account.

On the basis of these characteristics, monostarch phosphate, obtained through esterification of potato starch, was selected for analyses. It was characterized by relatively high resistance to the activity of amylolytic enzymes (reaching 55% and 70% when determined against α-amylase and glucoamylase, and 70% when determined according to the method of Englyst), compared to non-modified starch. Decreased susceptibility to the activity of amylases was reported in previous investigations of other authors [Sitowy & Ramadan, 2001]. This preparation demonstrated enhanced solubility (24%) and water absorption (67 g/g), both determined at 30°C, compared to non-modified potato starch.

Another preparation selected for biological analyses was monostarch phosphate obtained through esterification of soluble starch. It was characterized by increased susceptibility to the activity of amylolytic enzymes (reaching 50% and 75% when determined against α-amylase and glucoamylase, and 70% when determined according to the method of Englyst), compared to non-modified soluble starch. This preparation demonstrated also increased solubility (46%) and water absorption (25 g/g) determined at 30°C, compared to soluble starch. The available literature lacks data on the properties and resistance to amylase of monostarch phosphate obtained from soluble starch.

A product obtained from monostarch phosphate roasting with glycin and then subjected to the activity of a microwave field of 750W was the third preparation of resistant starch selected for biological analyses. It was characterized by an increased resistance to the activity of amylolytic enzymes, compared to non-modified potato starch (reaching 33% and 55% when determined against α-amylase and glucoamylase, and 70% when determined according to the method of Englyst). In addition, it displayed enhanced solubility (54%) and water absorption (71 g/g) determined at 30°C. There are no literature data that would refer to this preparation.

Analyses of resistant starch carried out on rats aimed at determining growth indices, biochemical and lipid parameters of blood and liver, the contents of mineral compounds, and the effect of RS preparations on the caecum.

Over a 4-week experiment, no unfavourable changes were observed in the appearance nor behaviour of animals fed experimental diets. Lower feed intake and statistically significantly lower body gains were reported for animals receiving preparations of resistant starch, compared to the control group (by 50–77%). The lowest body gain was observed in a group of rats administered with S2 starch-supplemented diet. This points to the presence of resistant starch in feed, since resistant starch fills the alimentary tract to the greatest extent than native starch and lowers the energetic value of a diet [Ito *et al.*, 1999]. Laxative effects, sometimes observed in feeding experiments on animals, were not reported in this study.

No differences were observed between mean weight of internal organs (liver and kidneys) in all groups examined, except for mean weight of kidneys in the group of S2 females (significantly lower compared to the control group).

There were no statistically significant differences between the levels of hematocrite, haemoglobin and glucose on serum of rats receiving all types of RS preparations. Investigations of other types of resistant starch pointed to decreased glucose levels in blood of experimental animals [Bornet *et al.*, 1989; Uled *et al.*, 1993; Muir, 1994].

A statistically significant reduction (by 30–40%) was observed for the level of triglycerides in serum of both female and male animals receiving diets supplemented with resistant starch, compared to the control group. No significant changes were, however, reported for the level of total cholesterol in serum of rats fed diets with RS preparations. Still, lower levels of HDL fraction were observed in males receiving diets with S1 and S3 starch preparations, compared to the control group. On the other hand, the average concentration of HDL fraction in serum of females fed a diet with S1 starch was significantly higher, compared to the other three groups.

Decreased serum levels of triglycerides and cholesterol were also observed in biological analyses of different RS preparations [Levrat *et al.*, 1996; Hsing-Hsian Cheng *et al.*, 2000; Lopez *et al.*, 2001].

It should be emphasized that the type of resistant starch, its content in a diet, composition and solubility in water determine the course of metabolic processes in the organisms of experimental animals, as well as exert diversified effects on biochemical and lipid parameters [Bednar *et al.*, 2001].

Average levels of triglycerides in livers of female and male rats as well as the level of total cholesterol in liver of males did not differ from the average levels of those parameters in the control group. On the contrary, a considerable decrease in total cholesterol level, compared to the control group, was observed in livers of females administered with S2 and S3 starches.

Blood sera of female and male rats were determined for the concentrations of 13 selected fatty acids, and their livers – for 15 fatty acids. Total concentrations of saturated, monounsaturated and polyunsaturated acids were calculated. The level of monounsaturated fatty acids was observed to decrease significantly (by several per cent) and that of polyunsaturated acids to increase significantly (by several per cent) in sera of both females and males receiving S1 and S2 starches, compared to the control group.

In livers of the experimental animals, the effect of added starches on the concentrations of selected fatty acids was diversified, depending on the sex of animals. An increase in the levels of saturated and monounsaturated acids, and a decrease in the level of polyunsaturated acids were observed in males administered with S2 and S3 starches, compared to the control groups. The examined groups of female rats receiving resistant starch preparations did not demonstrate any significant changes in the concentrations of saturated acids. Still, they were characterized by a negligible decrease in the level of polyunsaturated acids.

The addition of starch modified with phosphates caused a statistically significant increase in phosphorus concentration in serum of animals from groups S1, S2 and S3, compared to the control group. Statistically significantly higher levels of magnesium were reported in serum of females fed diets supplemented with RS preparations, compared to the control group. The average contents of calcium in serum of females from the control and S1 groups were significantly higher than those of females from groups S2 and S3. No differences were observed between magnesium levels in serum of rats and between iron levels in serum of both sexes.

The addition of resistant starch to feed resulted in a significant increase in the masses of caecal wall and caecal digesta. In groups of animals administered with resistant starch, the pH values of caecal digesta were also found to decrease significantly. Similar results, at the application of different types of resistant starch, were reported by other authors [Ebihara *et al.*, 1998; Stephen, 1994]. A decrease in pH of intestinal digesta stimulates the growth of beneficial microflora (*Bifidobacterium*, *Lactobacillus*) and reduced the number of pathogenic bacteria [Kleessen *et al.*, 1997; Silvi, 1999].

The presented research demonstrated changes in the concentrations of acetate, propionate and butyrate in the caecal digesta. These changes were found to highly dependent on the type of starch. The concentrations of the acids examined were observed to increase as well as to decrease. The intensity of the formation of short-chain fatty acids upon colonic fermentation is determined by the resistance, composition and properties of starch [Bednar *et al.*, 2001], and the presence of those compounds may affect lipid metabolism in the body.