Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta)

Ph.D. THESIS

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INTRODUCTION

The inadequate lifestyle and the wrong nutritional habits have been leading to the increasing number of overweight and obese people in the developed countries. Nowadays, about the half of the adult population and the 25% of the youngsters belong to the people struggling with extra weight.

The obesity itself is not only one problem as it can be responsible for the development of other serious diseases like type 2 diabetes mellitus, cardiovascular diseases or gastrointestinal disorders. There are several solutions in the treatment of obesity, the best way seems to be the diet and the plan of exercises adjusted to the personal metabolism. Several studies suggested a reduced fat intake and higher carbohydrate content of foods in the last decades. Even if the fat intake decreased from 42% to 34%, the number of overweight and obese children and adults increased significantly, suggesting that other dietary factors may have an equally important role in body weight regulation. Carbohydrates have traditionally been recommended in place of fat. In theory this is a logical approach to weight loss as carbohydrate has a relatively low energy density (17 kJ/g) compared to fat (37 kJ/g) and thus a high-carbohydrate diet is usually bulkier and more filling than a high-fat one. In addition, it was thought that fat tissue formation did not occur to any great extent in humans, and carbohydrates should contribute little to body fat stores. In some studies, however, it was shown that with massive carbohydrate overfeeding in healthy male subjects, net fat synthesis amounted to 170 g/day, of which 98% took place in adipose tissue. The typical Western diet contains many manufactured low-fat, high-carbohydrate foods that have a relatively high energy density. The standard dietary advice to reduce fat intake while increase carbohydrate intake, generally cause higher glycaemic effect of the diet. The grouping of foodstuffs according to their glycaemic effect can be a great help for the obese individuals in their weight management.

Accordingly the concept of glycaemic index (GI) was born to characterize the amount of liberated glucose in a two-hour long digestion period after the consumption of a starch based food containing 50 g available carbohydrate. GI is in connection with the fast and slow digestible carbohydrate content of food products. Low GI food may act an important role in the treatment of overweight and obesity. Accordingly the aim is to produce food with lower GI and energy density. One of the most promising solutions can be the addition of resistant starches into foods to decrease the GI and moreover increase the fibre content of products.

The term „resistant starch” (RS) has been defined by the European Flair Concerted Action on
Resistant Starch (EURESTA) as the starch or products of starch degradation that escapes digestion in the human small intestine of healthy individuals and may be completely or partially fermented in the large intestine as a substrate for the colonic microflora. Due to their health benefits and functional properties resistant starches play an important role in the nutrition. High resistant starch content in the diet may improve glucose and lipid metabolism, can reduce the risk of the development of type 2 diabetes mellitus, obesity, coronary and inflammatory bowel diseases, and gastrointestinal disorders. Resistant starches can be applied in starch-based products without causing significant changes in the sensory properties of the foodstuffs. Bread and pasta products belong to the basic foodstuffs having an important role in the human consumption. Bread making has played important role in the human culture for more than 4000 years and bread has been a popular staple food for ages. The nearly ubiquitous consumption of bread places it in a position of global importance in international nutrition. Bread is one of the major sources of carbohydrate, in the form of starch, in the human diet. Bread usually is rapidly digested and fast glucose release and absorption happens after its consumption. According to the fast glucose response, bread is described as a high-GI food playing remarkable role in the development of obesity and diabetes as well as weight gain. Based on the observation that bread produces a high GI, efforts have been made to develop bread products that induce lowered glycaemia and reduced demand for insulin.

Pasta products are also basic foodstuffs having an important role in the human food consumption. They can be easily prepared, handled, cooked and stored. The most important consumer attribute is the cooking quality of the pasta which includes the cooking time, water absorption, texture, taste and aroma of the cooked product. In recent years pasta has become even more popular due to its auspicious nutritional properties. Research has shown that sugars are progressively liberated from pasta during digestion, leading to low postprandial blood glucose and insulin response. Pasta is being regarded as a product with low glycaemic index (GI), playing important role in the treatment of obesity.

Several studies showed that fibres can be used in the preparation of both bread and pasta; however they caused significant changes in the texture and consumer value of the products. Accordingly our main aim was to investigate the properties of different resistant starches and to use them in starch-based products as fibre sources. After the literature overview the appropriate goals of the studies are summarized.
1 LITERATURE OVERVIEW

1.1 Starch as a major component in the human diet

1.1.1 Generally about the starch and its components

Starch has been extensively studied and discussed in the literature over the last two centuries, although the history of starch usage by man has been variously described for thousands of years. The ancient Egyptians (~4000 BC) and later the Romans used this substance as an adhesive whilst the ancient Greeks also used it in medical preparations. The popularity of starch grew in Europe around the 14th century, owing to its use for stiffening linen and starch was subsequently adopted for cosmetic purposes (Kaur et al., 2007). The starch granules and their properties have exercised scientific curiosity for hundreds of years. Van Leewenhoek used them as one of his subjects in his seminal work on microscopical discoveries and also made the first experiments on starch gelatinization. After his work on starch structure several studies appeared about the differences among the granules of different origin (Wang et al., 1998).

Moreover next to its proper stiffening and adhesive properties, the main role of starch is in the human diet as the dominant carbohydrate material of some foodstuffs (mainly tubers like potato and sweet potato as well as cereal based products) consumed regularly. It can be stated that starch is the major dietary source of carbohydrates stored as a reserve polysaccharide in plants being found as plastids in the leaf chloroplast and in the amyloplast of storage organs such as seeds, pulses and tubers (Sajilata et al., 2006, Wang et al., 1998). Most of the starch utilized worldwide comes from a relatively small number of crops, the most important being maize, potato, wheat and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago and mung beans. In Europe maize, wheat and potato are the main sources although in Scandinavian oats and barley are used as well.

In the food industry the main role of starch is its inclusion in the diet as a high-caloric food source and it is also used as gelling and pasting component in sauces, soups, dressings and spreads (Wang et al., 1998).

Much has been written about starch structure, properties, biosynthesis and degradation indicating the importance of the knowledge about the chemistry of this polysaccharide. Starch occurs in plants as water–insoluble granules, which display an enormous diversity of size.
(~1-100 µm) shape (round, lenticular, polygonal) size distribution (uni- or bi-modal), association of individual (simple) or granule clusters (compound), composition (α-glucan, lipid, moisture, protein and mineral content) and number of granules per plastid (one for maize, wheat, barley, several in rice and more than 30,000 in spinach) reflecting the botanical origin (Tester et al., 2004, Sólyom and Kudron., 1985). Some different starch granules are presented in Figure 1.

![Figure 1](image)

**Figure 1** Different starch granules: a, wheat; b, maize; c, barley; d, potato; e, rice

Starch granules are composed of two types of alpha-glucans, amylose and amylopectin which represent approximately 98-99 % of the starch dry weight. The two types of macromolecules are composed of a number of monosaccharides or sugar (glucose) molecules linked together with α-D-(1-4) and/or α-D-(1-6) linkages. Starches from most species are composed of about 20-30 % amylose and 70-80% amylopectin (Wang et al., 1998, Smith, 2001). In mutant lines of diploid species originating from crops such as maize, starches can be obtained with amylose contents in the range of 0% (waxy maize) to 84% (amylomaize) (Matveev et al., 2001).
Amylose (105 to 106 g/mol) is an essentially linear molecule consisting of long chains of \( \alpha\)-D-(1-4)-linked glucose units having a degree of polymerization (DP) up to DP 6000 and having a molecular weight of \( 1 \times 10^5 \text{ to } 1 \times 10^6 \). The chains can easily form single or double helices.

The remainder of the starch granule consists of amylopectin (107 to 109 g/mol), a branched polymer in which linear chains of \( \alpha\)-D-(1-4)-linked glucoses are joined together by \( \alpha\)-D-(1-6) linkages. The branch points are arranged so that the clusters of chains of about 12-20 glucose units occur at regular intervals of about 9 nm along the axis of the molecule. Chains of about 45 glucose units span two clusters and chains of about 70 glucose units span three clusters. The polymerization of the amylopectin has an average DP of 2 million, making it one of the largest molecules in the nature with the molecular weight of \( 1 \times 10^7 \text{ to } 1 \times 10^9 \) (Sajilata et al., 2006, Smith, 2001, Tester et al., 2004).

Based on X-ray diffraction four types of starch crystalline structures, designated as type A, type B, type C (Figure 2), and type V have been identified. These depend partly on the chain length making up the amylopectin lattice, the density of packing within the granules, and the presence of water. Although type A and type B are real crystalline modifications, type C and type V are mixed forms.

![Figure 2 Type A, B and C structure of starch (Sajilata et al., 2006)](image-url)
Type A: this structure has amylopectin of chain lengths of 23 to 29 glucose units. The hydrogen bonding between the hydroxyl groups of the chains of amylopectin molecules results in the formation of outer double helical structure. In between these micelles, linear chains of amylose parts are packed by forming hydrogen bonds with outer linear chains of amylopectin. This pattern is very common in cereals.

Type B: this structure consists of amylopectin of chain lengths of 30 to 44 glucose molecules with water inter-spread. This is the usual pattern of starches in raw potato and banana.

Type C: this structure is made up of amylopectin of chain lengths of 26 to 35 glucose molecules, a combination of type A and type B, which is typical in peas and beans.

![Figure 3 The cluster model of starches (Sajilata et al., 2006)](image)

The different types of starches are generally described as clusters (shown in Figure 3) which comprise the shorter A chains (80-90%) forming double helical structures and the longer B chains that form 10 to 20% of the whole cluster (Smith, 2001).

Type V: this type is a generic term for amyloses obtained as single helices co-crystallized with compounds such as iodine, dimetil-sulfoxide (DMSO), alcohols or fatty acids. Although such compounds are required for formation of the type-V structure, they are not systematically included in the amylose helix. In the case of amylose–lipid complexes, it is assumed that the aliphatic part of the lipid is included inside the amylose helix, while the polar group lies outside, being too large to be included. The amylose–lipid complexes can be crystalline or amorphous depending on the temperature at which they form (Buléon et al.,...
In the granules amylose molecules appear to be dispersed among amylopectin molecules and may be located primarily in the amorphous zones of the growing rings in granules and only a small amount is associated with the crystalline structure (Matveev et al., 2001). Accordingly amylose does not form part of the crystalline matrix as a constituent in the starch granule.

Another classification of starches can be based on their nutritional characteristics as it will be shown in the next section.

1.1.2 The classification of starches according to their nutritional properties

Starch must be completely depolymerised to glucose before it can be absorbed in the small intestine. Depolymerisation is affected by several digestive enzymes that cleave the glucosidic bonds. In monogastric species (like human) the main enzymes are amylases. When incubated with amorphous or highly dispersed starch, they act on both amylose and amylopectin in an endo fashion, releasing glucose, maltose and higher dextrins. The glucose is absorbed directly through the intestinal mucosa, whereas the oligosaccharides are acted upon membrane glucosidases such as glucoamylase (also called amyloglucosidas) (Annison and Topping, 1994). Based on the action of enzymes Berry (1986) classified the starches according to their behaviour when incubated with enzymes as follows:

**Rapidly digestible starch (RDS):** RDS consists mainly of amorphous and dispersed starch and is found in high amounts in starchy foods cooked or baked by moist heat, such as bread and potatoes. In this case starch granules are gelatinized and are more accessible to enzymatic digestion (Yue and Waring, 1998). It is measured chemically as the starch, which is converted to the constituent glucose molecules and digested totally within 20 min of enzyme digestion.

**Slowly digestible starch (SDS):** SDS is expected to be completely digested in the small intestine, but for some reasons it can be digested more slowly. This category consists of physically inaccessible amorphous starch and raw starch with a type A and type C crystalline structure, such as cereals and type B starch either in granule form or retrograded form in cooked foods. It is measured chemically as starch converted to glucose after a further 100 min of enzyme digestion.
**Resistant starch (RS):** RS was first coined by Englyst et al. (1982) to describe a small fraction of starch that was resistant to hydrolysis by α-amylase and pullulanase as well as other amylobitic enzymes. The term „resistant starch” (RS) has been defined by the European Flair Concerted Action on Resistant Starch (EURESTA) as the starch or products of starch degradation that escapes digestion in the human small intestine of healthy individuals and may be completely or partially fermented in the large intestine as a substrate for the colonic microflora acting as a prebiotic material (Faraj et al., 2004). RS is the starch not hydrolyzed after 120 min of incubation (Sajilata et al., 2006).

Resistant starches are subdivided into four fractions: RS1, RS2, RS3 and RS4 also called type I, II, III and IV (Englyst et al., 1992, Sajilata et al., 2006, Nugent, 2005, Asp et al., 1996, Themeier et al., 2005, Muir et al., 1995, Tungland and Meyer, 2002, Mun and Shin, 2006). The fractions differ according to the reason of the resistance as well as their origin and sources moreover their properties in the food technology.

**RS1:** represents starch that is resistant because it is in a physically inaccessible form occurring in partly milled grains and seeds as well as in some very dense types of processed starchy foods. RS1 is heat stable in most normal cooking operations and enables its use as an ingredient in a wide variety of conventional foods (Sajilata et al., 2006). These starch granules are only accessible after microbial hydrolysis of the cell wall (Themeier et al., 2005).

**RS2:** represents starch that is in a certain granular form and therefore resistant to enzyme digestion. This type encompasses semi-crystalline starches having a polymorph B or C type. These starches are only slowly or incompletely digested by α-amylases. Starch from green bananas, from potatoes or high-amylose wrinkled pea or maize starch belongs to this type (Themeier et al., 2005). The high-amylose maize starch is unique among other RS2 samples as it retains its structure and resistance during the processing and preparation of many foods (Nugent, 2005). The RS2 samples are generally sensitive to heat load and lose their resistance in cooking or baking by gelatinization (Muir et al., 1995, Yue and Waring, 1998).

**RS3:** represents the non-granular starch-derived materials that resist digestion. RS3 forms are generally formed during retrogradation of the starch, mainly its amylose component. During retrogradation starch molecules re-associate and can form tightly packed structures stabilized by hydrogen bonding (Haralampu, 2000). According to Leeman et al. (2006) both retrograded amylose and retrograded amylopectin could be expected to have an impact on different nutritional characteristics, though the nutritional consequences of promoting amylose retrogradation have been more extensively studied. RS3 occurs in cooked and cooled
potatoes, breads and generally forms during the cooling of gelatinized starch. RS3 is divided into two subtypes: RS3a (IIIa) containing crystalline amyllopectin and RS3b (IIIb) having a partially crystallized amylose network (Themeier et al., 2005). There is a strong relationship between the polymorphism of RS3 and its prebiotic behavior proved by small angle X-ray scattering (Shamai et al., 2003, Shamai et al., 2004). RS3 is of particular interest because of its thermal stability. This allows it to be stable in most normal cooking operations, and enables its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000).

**RS4**: represents the RS where novel chemical bonds other than α-D-(1-4) and α-D-(1-6) are formed. This type includes starches etherified, esterified, acid-modified, bleached, oxidized or cross-bonded with chemicals causing a decrease in the digestibility. The increase in the resistance depends on the starch base and the type and level of modification (Wolf et al., 1999, Nugent, 2005). Hydroxypropyl, acetylated and citrate starches are the most commonly used in the food industry (Han and BeMiller, 2007, Xie and Liu, 2004, Chuenkamol et al., 2007, Saartrat et al., 2005, Liu et al., 1999) and in some cases octenyl succinic anhydride starches (Bao et al., 2003) and butyrogenic resistant starches are used (Schmiedl et al., 2000) as well as glutarate starches spreading nowadays (Kim et al., 2008), however, it is not well known how the various types of RS4 are affected by digestion in the human body (Nugent, 2005). The four RS types are presented in **Figure 4**.

![Figure 4 The resistant starches: a, RS1; b, RS2; c, RS3; d, RS4 (Sajilata et al., 2006)](image-url)
1.1.3 Effects influencing the RS content of starch samples

The resistance of starches and their formation is influenced by several parameters and factors. These intrinsic factors can be as follows (Sajilata et al., 2006):

Inherent properties of starch

*Crystallinity of starch:* as it has been already mentioned, one of the causes of resistance to enzymes is the crystallinity of native type B granules as observed in the case of amylomaize (high-amylose) starch and also the encapsulation of starch within plant cell or tissue structures. Any treatment that eliminates starch crystallinity or the integrity of plant cells can increase the enzyme availability and reduce the content of RS, whereas recrystallization and chemical modification tend to increase the RS.

*Granular structure:* a large variability in susceptibility to amylases shown by raw starch granules also influences RS formation. Potato starch and high-amylose maize starch are known to be very resistant *in vitro* and incompletely absorbed *in vivo* while other cereal starches are slowly digested and absorbed *in vivo*. The smaller surface-to-volume ratio of the large potato granules and the higher amylose content in the maize starch granules play important role.

*Amylose/amylopectin ratio:* a higher content of amylose lowers the digestibility of starch due to positive correlation between amylose content and formation of RS. According to Åkerberg et al. (1998) there is a great influence of amylose/amylopectin ratio on the retrogradation process and accordingly on the RS3 formation in bread samples. The greater the content of amylose is, the more difficult the starch is to gelatinize and the more susceptible to retrogradation (Topping et al., 2003).

*Retrogradation of amylose:* the rate and extent to which starch may retrograde after gelatinization essentially depends on the amount of amylose present. Repeated autoclaving of starch may generate up to 10% RS. The retrogradation of amylose was identified as the main mechanism for the formation of RS in processed foods.

*Influence of amylose chain length:* Eerlingen et al. (1993) proved that the aggregation of amylose helices in a crystalline B-type structure can increase the RS content of a starch sample.
**Linearization of amylopectin:** during long low-temperature baking process the amylopectin chains go through linearization and it has been reported that this effect increase the RS formation (Sajilata et al., 2006).

**Heat and moisture**

Water content is an important factor that affects formation of RS. Repeated heat/moisture treatment is associated with a decrease in the hydrolysis of starches by amylolitic enzymes. Cooking increases starch digestibility while subsequent cooling leads to the formation of retrograded crystallites (Sajilata et al., 2006, Topping et al., 2003). According to Miyazaki and Morita (2005) heat-moisture treated maize starch can be a good source of dietary fibre in bread products.

**Food processing**

A lot of research has dealt with the technological and physicochemical behaviour of starch as the main constituent of many regularly consumed foodstuffs. The amount of starch reaching the human colon is greatly influenced by the nature of the diet and the ways in which food has been processed (Muir et al., 1995). Almost all food is heat-treated before being eaten so the investigations of the heat load and the changes in the structure of the starch granules are remarkably important. Production of starch based products often comprises a combination of shear and thermal treatment. It is well known that this treatment leads to molecular breakdown, depolymerisation, crystal melting and the disappearance of the granular structure (van den Elinde et al., 2004, Barron et al., 2001) The destructuring of the starch granules is shown in Figure 5 and Figure 6.
During thermal processing, the development of an amorphous starch phase first requires melting of crystalline structures which commences with the internal disorganization of starch granules. In the next phase fragmentation can be seen with the higher temperature and shear. At the end the starch crystalline structure totally disappears and an amorphous gel appears (Barron et al., 2001). According to van den Elinde et al. (2004) shear stress is the key parameter in the degradation of starch therefore this parameter has to be taken into account in the treatment more accurately (Figure 6).
Starch in processed foods can be in several structures dependent on the process. It can be unchanged, partially or wholly gelatinized, or partially retrograded. Gelatinization is the hydration process of the starches during moisture heat treatment (Björck et al., 1994). This phenomenon takes place when water content is high enough (water-starch ratio is over 0.75) (Turhan and Gunasekaran, 2002). Gelatinized starches show higher digestibility compared to raw starches and the hydrolysis is affected greatly by the extent of the heat load (Annison and Topping, 1994). Baking, pasta production, extrusion cooking, autoclaving and so forth are known to influence the yield of RS in foods. Boiling or pressure cooking may increase digestibility by 40-50 %, apparently as a result of gelatinization (Annison and Topping, 1994). Highly processed cereal flours and foods contain much lower levels of RS, averaging only about 1.5 to 8 % RS on a dry basis compared to the raw flour containing RS levels of about 20-30 %. Cooking under conditions of high moisture and temperature can significantly lower the RS content by disrupting the crystalline structure (Sajilata et al., 2006).

Although heat-treatment and different food processing steps cause a significant decrease in the RS content, the storage of starch-based products can lead to higher RS levels. It is possible due to the retrogradation process where gelatinized starch is transformed into a more ordered or crystalline state. This process can be a good method for preparing RS3 starches (Åkerberg et al., 1998).

**Interactions of starch with other components**

*Protein:* starch-protein interaction has been believed to reduce RS contents as observed in case of potato starch and added albumin when autoclaved and subsequently cooled at -20°C (Escarpa et al., 1997). In some processed foods, protein may encapsulate the starch granules. According to Hoebler et al. (1999), the physical barrier created by the protein network in cereal based products limits the accessibility of starch to amylase and delays *in vitro* starch hydrolysis resulting in increased resistance.

*Dietary fibre:* according to Escarpa et al. (1997) insoluble dietary fibre constituents such as cellulose and lignin had minimal effects on RS yields. Guar and xanthan gums as two water-soluble, non-ionic polysaccharides influence the gelatinization properties of starches and therefore they affect the retrogradation process as well (Achayuthakan and Suphantarika, 2008).
Enzyme inhibitors: some substances inhibit α-amylase activity in vitro such as proteins or glycoproteins present in legumes and in cereals; antinutrients such as tannin, polyphenols, phytic acid and lectins; hydrolysis products especially maltose and maltotriose (Asp et al., 1996, Sajilata et al., 2006).

Ions: according to Escarpa et al. (1997) in potato starch gels calcium and potassium constituents caused decrease in the yields of RS probably due to the prevention of formation of hydrogen bonds between amylose and amylopectin chains caused by the absorption of these ions.

Sugars: the role of sugars on the formation of RS in starch gels (RS3) was studied by Eerlingen et al., (1994). Results showed that sugars influenced the retrogradation process only in a high concentration (starch-water-sugar ratio of 1:10:5 w/w).

Lipids, emulsifiers: according to Asp et al. (1994) and Crowe et al., (2000) amylose-lipid complexes had a reduced digestibility compared to free amylose. The extent of this decrease depends mainly on the type of lipid (monoglycerides form complexes highly resistant to amylolysis) (Sajilata et al., 2006) and the amylose-amylopectin ratio.

1.1.4 The analysis of starches and resistant starches

There are several methods in the analysis of starches according to their pasting, rheological, thermal, morphological, botanical origin and digestibility characteristics.

1.1.4.1 Determination of the physicochemical properties of starches

There are big differences among the starches in their pasting and rheological properties studied by rapid visco analyser (RVA) method and dynamic rheometry as well as in their thermodynamics measured by differential scanning calorimetry (DSC). According to Gelencsér et al. (2008) resistant starches were not able to gelatinize in the standard RVA procedure independently from the type of the RS. Gelatinization properties of starches depend generally on the type, granular structure, botanical origin, amylose/amylopectin ratio (Sajilata et al., 2006) and in the case of potato starches on the phosphorous content (Zaidul et al., 2007). High-amylose content in the starches can lead to lower gelatinization affinity using the standard RVA procedure (Jane et al., 1999). Probably due to its chemical modification, RS4
starches can not gelatinize either in the RVA method as it has been proved by Xie and Liu (2004) in the case of citrate starches. The native starches can be differentiated by botanical origin using the RVA, the resistant starches, however, can not be described by the origin in this method. Hagenimana et al. (2005) have studied starches using dynamic rheometry and proved that first of all amylose content can influence the rheological properties of starch samples. The structures of the carbohydrates as well as the gelatinization and retrogradation process of starch gels can be followed by Raman spectroscopy and Fourier transform infrared spectroscopy as well (Fechner et al., 2005, Zhbankov et al., 1997, Schuster et al., 2000).

Differential scanning calorimetry (DSC) seems to be able to measure the changes and differences in RS types during a heating procedure (thermal properties). The transition and enthalpy of gelatinization of starches can be determined using DSC related to characteristics of the starch granule, such as degree of crystallinity and amylose-amylopectin ratio (Singh and Singh, 2001, Matveev et al., 2001). According to Tananuwong and Reid (2004) there are two endothermic peaks on the DSC curves related to the gelatinization process influenced by several parameters. In the case of RS samples only one peak or no peaks were detected indicating different behaviour in the method compared to native starches (Hagenimana et al., 2005, Gelencsér et al., 2008).

The thermal properties of starches can be furthermore determined by near-infrared (NIR) spectroscopy used mainly in cereal breeding programmes (Bao et al., 2007). The NIR system moreover is accurate to measure the ratio of amylose to amyllopectin, the physical and chemical properties as well as pasting characteristics of starches (Fertig et al., 2004, Lu et al., 2006).

The morphology of starches can be evaluated by scanning electron microscopy which is able to differentiate starches by genotype and pasting properties. Laser light scattering has been used to characterize starch granule diameter, mainly in the case of spherical granules. (Singh and Singh, 2001). Small angle X-ray scattering is to investigate the colloidal structure of starches related to the crystalline structure. The retrograded RS3 starches can be well determined by this method (Shamai et al., 2004, Millan-Testa et al., 2005).

The botanical origin of the starches can be determined on several ways. The identification usually depends on the microscopic shape character of the granules because their chemical composition is very similar. Therefore scanning electron microscopy and light microscopy as well as X-ray scattering are spread (Shamai et al., 2004, Millan-Testa et al., 2005). Some other methods are also available to evaluate the starches according to their origin. One of
them is the Raman spectroscopy which is able to classify starch samples and identify their modifications (RS4) if there are any (Dupuy and Laureyns, 2002). Another method is the glucose biosensor based on glucose oxidase and amylglucosidase which can properly and effectively classify starch samples according to their origin (Chough et al., 2006).

1.1.4.2 Determination of the enzymatic digestibility properties of starches

There are different in vitro and in vivo methods in the determination of digestibility characteristics of starches. Although in vitro methods are preferred (simplicity, measurements under laboratorial circumstances) the extent of in vivo human and animal studies are still very high. It is mainly due to the real matrix in which starches can be digested and absorbed according to the metabolism.

There are specific methods to determine RS in foods. Direct methods quantify RS in the residues obtained after removing digestible starch (Berry method) (Berry, 1986), while indirect methods determine RS as the difference between total starch and digestible starch (Englyst method, Goñi method) (Englyst et al., 1987, Goñi et al., 1996). Some of the methods use only amylases, whereas others use proteolytic enzymes in combination with amylases (Goñi et al., 1997). Resistant starches are typically quantified as part of the total dietary fibre (TDF) content (Englyst et al., 1987). The predominant assays for the regulatory determination of TDF are AOAC and AACC methods. They are identical. Both old (AOAC 985.29 and AACC 32-05) and new (AOAC 991.43 and AACC 32-07) methods in the first step extract lipid, digest carbohydrates and protein enzymatically and arrive at the remaining non-digestible fibre content of sample gravimetrically. The difference between the old and new methods is in the buffer system and washing solutions (Haralampu, 2000). Englyst et al. (1992) have proposed an analytical method for rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) and their method is used in most studies still now (Megazyme, 2004).

Muir and O’Dea (1992 and 1993) made another method for the determination of RS in food products using chewing by subjects in the procedure. This method is more complicated, the results are at the same time more precise and reproducible.

Different methods are used to analyze RS in vivo. One of the ways to assay RS physiologically is to determine starch in the undigested ileal content (the content of the ileum). Terminal ileal samples can be recovered by intubation or from ileostomy bags. The
classic way to substantiate starch digestion is by measuring the glycaemic index (GI) of foods which is a strong function with the starch digestibility. The measurement of glycaemic index includes blood sampling in certain time periods and the analysis of blood sugar content. Determination of breath hydrogen and methane (breath test) or short chain fatty acids in the gut can also be used as a semi quantitative measurement for RS (Sajilata et al., 2006, Asp et al., 1996). The in vivo results are influenced by several factors such as transit time, physical inaccessibility, amylase concentration, and the presence of other food components which determine the amount of starch that reaches the terminal ileum (Annison and Topping, 1994).

1.1.6 Health properties of resistant starches

Resistant starches are defined as a part of dietary fibres according to their indigestion property in the human small intestine. According to being dietary fibre, resistant starches have several health benefits which have to be known and investigated. The health benefits of resistant starches are listed in Table 1.

**Table 1 Health properties of resistant starches (Nugent, 2005)**

<table>
<thead>
<tr>
<th>Potential physiological effects</th>
<th>Conditions where there may be a protective effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improve glycaemic and insulinaemic responses</td>
<td>Diabetes, impaired glucose and insulin responses, the metabolic syndrome</td>
</tr>
<tr>
<td>Improved bowel health</td>
<td>Colorectal cancer, ulcerative colitis, inflammatory bowel disease, diverticulitis, constipation</td>
</tr>
<tr>
<td>Improved blood lipid profile</td>
<td>Cardiovascular disease, lipid metabolism, the metabolic syndrome</td>
</tr>
<tr>
<td>Prebiotic and culture protagonist</td>
<td>Colonic health</td>
</tr>
<tr>
<td>Increased satiety and reduced energy intake</td>
<td>Obesity</td>
</tr>
<tr>
<td>Increased micronutrient absorption</td>
<td>Enhanced mineral absorption, osteoporosis</td>
</tr>
<tr>
<td>Adjunct to oral rehydration therapies</td>
<td>Treatment of cholera, chronic diarrhoea</td>
</tr>
<tr>
<td>Synergistic interactions with other dietary components, e.g. dietary fibres, proteins, lipids</td>
<td>Improved metabolic control and enhanced bowel health</td>
</tr>
<tr>
<td>Thermogenesis</td>
<td>Obesity, diabetes</td>
</tr>
</tbody>
</table>
RS, by escaping digestion in the small intestine, has few interactions with other components of the upper gastrointestinal tract. It is fermented in the large intestine resulting in the production of such fermentation products as carbon dioxide, methane, hydrogen, organic acids and short chain fatty acids (SCFA-s) (Nugent, 2005). Moreover resistant starch has significant physical effects in the gut and in addition through the fermentation it is a major determinant of large bowel function and bowel habit. Its physical properties in the small bowel effect lipid absorption, and the glycaemic response and it has a modest effect on appetite as well (Cummings et al., 2004, Tungland and Meyer, 2002). SCFA-s are the metabolic products of anaerobic bacterial fermentation of polysaccharides, oligosaccharides, protein, peptide and glycoprotein precursors in the large intestine. The principal SCFA-s are butyrate, propionate and acetate known as the respiratory fuel of cells lining the colon (colonocytes). They increase colonic blood flow, lower luminal pH and help prevent the development of abnormal colonic cell populations. SCFA-s are commonly used as markers of fermentation and colonic health. Resistant starches can increase the production of these important SCFA-s and therefore may help improve colonic health. In animal studies RS feeding increased the caecal and faecal production of total SCFA and also the individual concentrations of propionate, butyrate and acetate. RS2 was also proved to increase the butyrate production in rats and humans (Nugent, 2005). Butyrate is known to have beneficial effects on the reduction of risk factors involved in the etiology of colon cancer and adenoma development moreover it is the prime energy substrate of the colonocytes. There is a strong relationship between the RS content of food samples and the gut health properties regarding butyrate production (Brouns et al., 2002). Moreover SCFA stimulate cation (sodium, potassium, calcium) uptake in the proximal colon and through their action on muscular activity and blood flow in the colon may directly reduce the severity of diarrhoea. A large number of animal (generally focused on rats, pigs and mice) and human studies have attempted to investigate the effects of RS on colonic function. According to the studies it could be concluded that RS appears to have a protective effect on markers of colonic function indicating a favourable effect on the bowel health (Nugent, 2005). Birkett et al. (1996) showed that RS has a significant effect on the fermentation of protein in the human colon as well. Most notable was the effect of high-RS diet on the excretion of the potentially harmful byproducts of protein metabolism such as ammonia and phenols. The RS resulted in a reduction in the concentration
of these molecules. RS is known to affect lipid metabolism based on studies in rats where reductions in a number of measures of lipid metabolism have been observed. These include total lipids, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), tryglicerides and tryglcieride-rich lipoproteins. RS was reported to lower plasma and liver cholesterol levels in obese rat species (Nugent, 2005, Lopez et al., 2001), this effect was not proved in the human body (Asp et al., 1996), however it is certain that some forms of dietary fibre can lower blood cholesterol in man leading to lower risk in the development of coronary heart disease (Cummings et al., 2004). During high-RS diet, faecal concentrations of total neutral sterols decreased by 30 % and faecal concentrations of 4-cholesten-3-one decreased by 36 % according to Hylla et al.(1998) proving the effects of RS on the bacterial metabolism in the human colon and its role in cancer prevention. According to Cassidy et al. (1994) there is a significant negative correlation between the consumption of starch and with it resistant starch on colorectal cancer incidence. Some experimental studies suggest the intake of \( \geq 20 \text{g/day} \) RS to obtain some of the bowel-related benefits (Baghurst et al., 1996).

RS appears to function as a probiotic, prebiotic and symbiotic interacting with the colonic microflora. In vitro studies have shown that RS may physically associate with several Bifidobacteria species protecting them from attack during food preparation and storage. Therefore RS was described as a culture protagonist and was combined with Bifidobacteria or Lactobacillus species by encapsulation in yoghurt (Nugent, 2005, Topping et al., 2003, Charalampopoulos et al., 2002, Mattila-Sandholm et al., 2002).

RS-rich foods release glucose slowly and therefore lower insulin response, greater access to and use of stored fat and, potentially, a muted generation of hunger signals are expected. With RS-s it would be possible to manage clinical conditions, such as diabetes and improved glucose tolerance as well as the treatment of obesity and weight management (Nugent, 2005, Cummings et al., 2004). RS can also be a protective factor against the development of type II diabetes, obesity, coronary diseases, gastrointestinal disorders, and inflammatory bowel diseases (Meyer et al., 2000; Tungland and Meyer, 2002, Thornburn et al., 1987).

The glucose release after the consumption of a starch based product is generally dependent on the type, form and amount of starch present in the food. Accordingly it can be stated that the glycaemic response is in a strong relationship with the non-digestible starch content of samples. To be able to range the foods according to their digestibility the concept of glycaemic index (GI) has been developed. The main aspects of GI are discussed in the next chapter.
1.2 Glycaemic index and its importance in the human diet

1.2.1 The concept of glycaemic index (GI)

For over half a century it has been postulated that an increase in blood glucose was less pronounced after consumption of starchy foods (polysaccharides) than after consumption of foods containing simple carbohydrates (mono-or disaccharides). However, review of the literature yields no clue regarding the source of this alleged difference. Jacobsen in 1913 was able to show that glycaemia increase in healthy subjects followed the same path after a starchy meal with plenty of bread and potatoes, as after a meal rich in simple sugars. In the early 70s several teams also found that identical quantities of carbohydrates obtained from different foods gave different postprandial glycaemic responses. These observations inspired Jenkins et al. (1981) to introduce the idea of the glycaemic index (GI) of foods, for clarification and quantification purposes (Bornet et al., 1997, Colombani, 2004). The glycaemic index (GI) is a ranking of foods based on the postprandial blood glucose response compared with a reference food. The GI is usually defined as the area under the glycaemic response curve during a 2-hour period after consumption of 50 g carbohydrate from a test food, and values are expressed relative to the effect of either white bread or glucose (Roberts, 2000). The definition has been changed nowadays and in the tests 50 g available carbohydrate has to be used (Brouns et al., 2005). The GI concept was originally introduced as a means of classifying different sources of carbohydrate and carbohydrate-rich foods in the diet, according to their effect on postprandial glycaemia. It was proposed that the GI of foods was needed to supplement tables of nutrient composition in prescribing diets for individuals with diabetes (Brand-Miller, 1994). Moreover the use of GI and the information about its meaning could help guiding food choices of the consumers (Brand-Miller et al., 2002). This new classification of carbohydrate-foods is important in that the chemical content of a food is now replaced with its biological response. Measuring the kinetics of glucose levels from food intake thus produces a glycaemic index that is a fair reflection of the bioavailability of the carbohydrate under examination (Bornet et al., 1997).
The first GI table was published by Jenkins et al. (1981) reporting the glycaemic effect of 62 carbohydrate foods. Foster-Powell and Brand-Miller (1995, 2002) published an expanded table in 1995 and then in 2002 containing nearly 1300 data representing 750 different food types. In the table there are three groups according to the extent of the measured GI. High-GI foods (GI is above 70) are those that have the highest peak circulating glucose in the 2-hour period following food ingestion and the highest area under curve for the increase in blood glucose above fasting baseline. White bread samples, cooked potatoes, cookies, cakes and overcooked vegetables represent this category. On the other hand, low-GI foods (GI is below 50) are those that cause lower peak glucose, demonstrate a smaller area under the curve for the increment in blood glucose in the 2-hour postprandial period, and have a lower risk of causing relative hypoglycaemia. Raw vegetables, partly milled grains and pasta samples represent typically low-GI products (Roberts, 2000). The medium GI stands between high and low GI foods. The two main types (low and high GI) are presented in Figure 7 and a comparison of different food products in Figure 8.

![Figure 7](image-url) The meaning of low and high GI (Bornet et al., 1997)
The rate of glucose entry into blood and the duration of elevated blood glucose are known to induce many hormonal and metabolic changes that may affect health and disease parameters. In this respect, low-GI foods were often found to induce benefits on risk factors for certain chronic diseases. Dietary guidelines recommend the consumption of low-GI foods especially for elderly people helping to maintain their health. Low-GI foods can play important role in the treatment of type II diabetes mellitus, protect against the development of obesity, colon cancer and breast cancer. Several studies proved the positive effects of low-GI diet in the treatment of diabetes (Olausson and Kilander, 2008, Colombani, 2004, Brand Miller, 1994, Jenkins et al., 1988, Meyer et al., 2000). In addition, higher blood HDL-cholesterol concentrations were observed in patients consuming low-GI diets. Indeed, several studies have shown that the dietary GI is a good predictor of HDL concentrations in the healthy population (Brand-Miller et al., 2002, Frost et al., 1999). Laboratory studies examining the short-term satiating effects of foods have shown that low-GI foods are relatively more satiating than are their high-GI counterparts (Brand-Miller et al, 2002). Satiation is defined as the sensation of fullness that develops during the progress of a meal and contributes to meal

**Figure 8** Glycaemic indices of different food samples (Bornet et al., 1997)
termination, whereas satiety is defined as the sensation of fullness between one meal and the next, and relates to the return of hunger. High-GI carbohydrate foods promote a more rapid return of hunger and increases subsequent energy intake (Roberts, 2000). The GI concept has often been criticized as being inapplicable to everyday settings because it does not take into account the serving size actually eaten. This shortcoming has been eliminated with the glycaemic load (GL) concept. The GL is the product of the GI and of the food and sizes of typical servings (Colombani, 2004). The GI table also contains the GL data observing the typical serving size of a food product (Brand-Miller et al., 2002, Colombani, 2004).

### 1.2.2 Glycaemic index, carbohydrates and obesity, is there a connection?

Several studies suggested a reduced fat intake and higher carbohydrate content foods in the last decades. Even if the fat intake decreased from 42% to 34%, the number of overweight and obese children and adults increased significantly, suggesting that other dietary factors may have an equally important role in body weight regulation (Jacobs and Wood, 2004). Carbohydrates have traditionally been recommended in place of fat. In theory this is a logical approach to weight loss as carbohydrate has a relatively low energy density (17 kJ/g) compared to fat (37 kJ/g) and thus a high-carbohydrate diet is usually bulkier and more filling than a high-fat one. In addition, it was thought that fat tissue formation did not occur to any great extent in humans, and carbohydrates should contribute little to body fat stores. In some studies, however, it was shown that with massive carbohydrate overfeeding in healthy male subjects, net fat synthesis amounted to 170 g/day, of which 98 % took place in adipose tissue. The typical Western diet contains many manufactured low-fat, high-carbohydrate foods that have a relatively high energy density (McMillan-Price and Brand-Miller, 2004). The standard dietary advice to reduce fat intake while increase carbohydrate intake, generally cause higher glycaemic effect of the diet. Both the quantity and quality of the carbohydrates influence postprandial glycaemia and the interaction between them may be synergistic. A typical Western, high-carbohydrate diet based on high-GI foods such as potatoes, breads and low-fat cereal products is digested and absorbed rapidly, resulting in a high glycaemic load and increased demand for insulin secretion. In insulin-resistant persons who consume high-GI foods, postprandial hyperglycaemia and insulinemia are magnified, possibly contributing to
the development of type II diabetes mellitus. On the other hand, low-GI high-carbohydrate foods may maintain insulin sensitivity and increase the weight loss potential. Low-GI foods may benefit weight control in two ways: 1, by promoting satiety and 2, by promoting fat oxidation at the expense of carbohydrate oxidation (Brand-Miller et al., 2002). The treatment of obesity and overweight is possible by using low-GI diet in clinical studies according to Nikolić et al. (2004). It was found that the optimum amount of ingested carbohydrate designed to lower insulin response, improve access to stored fuels in the cells, decrease hunger and promote weight loss when abundant quantities of vegetables, fruits and legumes with moderate amounts of meat and less refined grain products, potato and concentrated sugar were consumed indicating a strong relationship between glycaemic index and carbohydrates (Jacobs and Wood, 2004, Ludwig, 2000).

Rapidly digested starch (starch in solution, gelatinized starch, partially degraded starch, and finely milled grains) cause glycaemic and insulin responses similar to those produced by an equivalent amount of glucose (Morris and Zemel, 1999). At the same time high resistant starch concentration in the diet—which is frequently associated with low-GI foods—may also improve glucose and lipid metabolism because the addition of RS to the diet increases the total amount of indigestible carbohydrates in the diet (Liljeberg et al., 1999). According to Raben et al. (1994) the intake of RS resulted in significantly lower postprandial plasma glucose, lactate, insulin and satiety hormone responses. The control of starch digestion with the introduction of RS into the diet is fundamentally important for glycaemic control (Cummings et al., 2004). Efforts have been made to use RS starches in low-GI products; however the amount of starch reaching the colon and the GI of a food product is greatly influenced by several parameters. The effects influencing the digestibility of RS have been discussed previously; the factors affecting the GI are being discussed in the next chapter.

1.2.3 Factors effecting the glycaemic index

A lot of factors are well known to have great impact on the liberated glucose level from a consumed food in the human body. The first important factor has already been discussed, and it was concluded that the higher amount of rapidly digestible starch leads to higher glucose concentration, while the higher amount of slowly digestible or resistant starch may cause lower blood glucose level. However there are a lot of other factors which influence the predicted GI of a food sample.
Measurement

Lots of studies have dealt with the development of GI methodology in vivo. The problem is mainly the number of subjects (representative population), the health properties of the subjects as well as their age and individual’s metabolism. Moreover, the blood sampling (venous or capillary blood) also shows strong influence on the GI. The method accepted nowadays was created by Brouns et al. (2005) giving several advices and a standardized procedure for the in vivo determination of GI. The in vitro GI measurements based on the estimation of starch digestibility are different according to the enzymes used in the tests (mono- or multienzyme procedures) as well as the circumstances during the hydrolysis. Goñi et al. (1997) made a procedure to estimate glycaemic index according to starch hydrolysis, their method, however, was several times modified, over thought or compared to other methods thought to be better (Granfeldt et al., 2006, Germaine et al., 2008). Nowadays, the methods of Holm and Björck (1992), Goñi et al. (1997) as well as Germaine et al. (2008) are used in the in vitro GI tests.

Blood sampling

There are two ways of blood sampling in the determination of in vivo GI. Capillary and venous blood are usually taken and analysed. According to Foster-Powell et al. (2002) capillary blood sampling would be better compared to the venous blood sampling. Although, capillary and venous blood glucose values have been shown to be highly correlated, it appears that after food consumption the glucose concentrations change to a greater degree in capillary blood samples than in venous blood samples. Therefore, capillary blood may be a more relevant indicator of the physiological consequences of high-GI foods.

Food processing

The modern methods of food processing affect the rate of starch digestion in foods and the amount of starch reaching the colon, subsequently blood glucose profile for starch-based products. Cooking, baking, extrusion cooking or other processing with higher temperature and pressure increase the availability of the starch to amylase causing rapid digestion after consumption, leading to fast glucose release in the blood (Brand et al., 1985, Muir et al., 1995). Additionally, the different brands or food manufacturers use different methods or ingredients in the processing of the same food, therefore the estimated GI can vary.
significantly. The glycaemic index table thus contains the names and suppliers of foods as well (Foster-Powell et al. 2002).

**Metabolism**

In addition to the rate of carbohydrate digestion, food mediated effects on both gastrointestinal events and post-absorptive metabolism can influence the GI. Gastric emptying is affected by food particle size and fat content as well as by viscous fibre, which also limits enzymatic hydrolysis in the small intestine by restricting access to the food components. Post-absorptive factors that can influence GI include the identity of the sugar moieties, which are metabolised differently and the insulinotropic effect of protein, which can increase the clearance rate of circulating glucose. Therefore the GI values do not represent the direct measure of carbohydrate absorption from the small intestine. Rather, the GI values are determined by the combined effect of all the properties of food that influence the rate of influx and removal of glucose from the circulation (Englyst et al., 2003).

**Prefeeding**

The GI of the foods is greatly influenced by the foods consumed previously. A high-GI evening meal can have effects on the GI of the breakfast (Granfeldt et al., 2006) and a low – GI breakfast can influence the GI and glucose tolerance at a subsequent lunch in healthy subjects (Liljeberg et al., 1999). Moreover it has been proved that a small dose of fructose prefeeding, consumed 30 to 60 min before a high-GI, starchy food reduces the glycaemic response compared with either immediate or no fructose treatments. This finding may have practical applications because there is a small amount of fructose in fruits, such as apple consumed regularly (Heacock et al., 2002).

According to the influencing factors, the determination of GI is rather difficult. Several methods are available but it is not guaranteed that one of them is the best and generally used. The different GI methods are shown and compared in the next chapter.

**1.2.4 Determination of glycaemic index**

Meanwhile the definition of GI is quite simple, its determination cause big problems among laboratories and scientists. Sometimes the GI values for similar foods vary between
laboratories because of the method used for determining the carbohydrate content of test foods as the first step of the GI prediction. Most researchers rely on food-composition tables or food manufacturer’s data, whereas others directly measure the starch and sugar contents of the foods. Another big difference is the type (*in vitro* or *in vivo*) of measurements. It was shown that GI values measured *in vivo* can be significantly different for the same food measured *in vitro*. However measuring the GI *in vitro* has been suggested as a cheaper and less time-consuming method, some researchers still insist on the *in vivo* determination and rely only on the human studies. Accordingly there is a significant need to prepare *in vitro* methods correlated with the *in vivo* results well (Foster-Powell et al., 2002).

### 1.2.4.1 In vitro methods for predicting the GI

Measurement of GI requires the use of human subjects for evaluation of the 2h blood glucose response after food intake. This resource intensive, physiological test is not suitable for new food product development or quality insurance. *In vitro* testing has been proposed as a faster, more cost effective method of screening food products for their GI during product development, prior to selection of optimised products for *in vivo* GI testing. Due to the complexity of the human digestive process, no *in vitro* test has been identified which can replace human GI testing, although *in vitro* starch digestibility methods are relatively successful at predicting the GI of high-starch foods. There are currently no international standards for *in vitro* starch digestibility testing and methods vary widely. Key differences between published methods include variations in the initial food breakdown procedure (chewing, mincing, milling, homogenizing), amounts and types of enzymes used (in some cases only amylase is used (O’Dea et al., 1981), in others it is combined with proteases (multi-enzyme methods) (Goñi et al., 1997) and incubation using non-restricted (test-tube), versus restricted (dialysis) systems. Mechanical mincing is reported to give a similar degree of food breakdown to chewing but with less variability confirming its use as the replacer of human chewing for routine *in vitro* testing (Germaine et al., 2008, Goñi et al., 1997). Germaine et al. (2008) suggests the method with mincing in a non-restricted (test-tubes) system using multi-enzymes.

In the method of Goñi et al. (1997) the *in vitro* procedure simulate starch enzymatic digestion. In the first step a protease (pepsin) treatment is carried out to avoid or destroy protein-starch interactions, in the next step \(\alpha\)-amylase is added to hydrolyse starch, finally
Amyloglucosidase should release glucose from the starch hydrolysis products. According to this method, the hydrolytic process can be approached by first order kinetics and described by the nonlinear, first order equation rate. Using the kinetic parameters, GI can be calculated. According to Trout et al. (1993) it is also possible to predict the GI from the compositions and macronutrient content of foods. In the first step, the main source of starch has to be identified, whether it is from legume, grain or a tuber and from what species and cultivar. A second step is to analyze methods of food preparation. A third step is to adjust published GI values of a similar food for such factors as special characteristics of the plant cultivar; degree of ripeness when harvested; reduction of particle size before, during or after cooking; and degree and manner of cooking. This calculation gives very inaccurate GI but may often be useful if measurements are not possible.

It can be concluded that the *in vitro* measurements are great predictors of the GI; however, for the appropriate value of GI, *in vivo* studies are also needed.

### 1.2.4.2 In vivo methods for predicting the GI

The *in vivo* measurements are based on the definition of GI or it could be also said that the definition of GI is based on *in vivo* measurements. Accordingly, the GI is predicted from the blood glucose response in a 2 hour long period of time after the consumption of a starchy food containing 50 g carbohydrates. This procedure seems to be simple, however, there are differences in the testing methods including the use of different types of blood samples (finger-prick capillary or venous), different experimental time periods (longer than 2 hours, different for healthy and diabetic individuals according to Wolever et al., (1991)), and different portions of foods (50 g of total rather than of available carbohydrate) (Foster-Powell et al., 2002). There are differences in the evaluation of the glycaemic response curves as well; therefore, the importance of a standardized method has had strong importance. Brouns et al. (2005) achieved the glycaemic index methodology, used as a basis in the *in vivo* studies nowadays. Based on the results of studies carried out previously they gave suggestions for all of the steps and calculations in the process:

**Number of subjects:** the inclusion of ten subjects provides a reasonable degree of power and precision for most purposes of measuring GI. The number of subjects can be increased if the aim of the study is to detect small differences in GI or when greater precision is required.
Test number: it is recommended to repeat the trials of the reference food at least once, to obtain at least two values in each subject. The test food might be measured only once.

Subject status: for routine testing, healthy human volunteers are recommended, however, the subject characteristics do not appear to have significant effect on mean GI values.

Reference food: as some subjects can get nauseous after taking a concentrated glucose drink in the morning after an overnight fast, it is recommended to use reference food other than glucose (such as white bread).

Meal volume, composition, consumption time: the test should be carried out in the morning (generally before 10:00, after a 10-14 h overnight fast). The test food (also reference) is calculated according to its carbohydrate content. For bread referent, 250 ml fluid (generally water) is allowed to drink, in the case of glucose 50 g of the sugar is diluted in 250 ml water. Fluid ingestion is advised to take place within 5-10 min, while solids and semi-solids should be ingested within 10-20 min, depending on the type and taste of food. The first blood sample should be taken exactly 15 min after the first bite of the food or first sip of the drink.

Carbohydrate basis and dose: it is recommended that the measurement of GI of test foods is based on comparing equivalent amounts of available carbohydrate, rather than total carbohydrate or serving size. The testing of 50 g available carbohydrate loads is recommended. In the case of foods with moderate carbohydrate density, it is justified to lower the carbohydrate load to 25 g to avoid an unrealistically large meal size.

Preparation of subjects: in practice there is no need for rigorous control of exercise, smoking or diet on the day before the test. It is recommended that the evening before a test each subject should consume a meal of choice and repeat that meal before each test. Smoking should not be allowed on the day of the test. The test should be done in 4 month, the reference test should be carried out at the beginning and a repetition should take place every 6-8 weeks.

Blood sampling: both finger-prick capillary and venous blood can be analyzed. In the case of capillary blood taking the sensitivity of subject has to be taken into account, while in the case of venous blood sampling arterialised venous blood is preferred to normal venous blood. The sampling schedule in healthy subjects should be as follows: fasting (0) and at 15, 30, 45, 60, 90 and 120 min after starting to eat the test meal.

Calculation of GI: incremental AUC (area under curve) is the method recommended by the Food and Agriculture Organization (1998) and the method used for most calculations of GI up to the present time. The incremental AUC is the area over the baseline under the curve, ignoring area beneath the baseline. The baseline is defined as the blood glucose at the start of
the measurement (Figure 9). The GI can be calculated from the incremental AUC of the test food \((f)\) and the incremental AUC of the reference food \((r)\) as the ratio of \(f:r\) for all subjects. The individual values of \(f:r\) are then averaged over all subjects to give the GI of the test food.

![Figure 9 Incremental AUC](image)

**Figure 9** Incremental AUC: the areas of 1+2+3+4+7 are calculated, 5 and 6 are ignored

(Brouns et al, 2005)

It can be concluded that there are a lot of factors that should be taken into account when GI is to be predicted. This method, however, seems to be worldwide accepted and used nowadays in the analysis of starchy foods such as pasta and bread etc. In the next section the nutritional properties and glycaemic characteristics of these two typical and regularly consumed products are summarized.

1.2.5 Bread as a typical high-GI foodstuff

Bread is one of the major sources of carbohydrate, in the form of starch, in the human diet. During baking, starch undergoes a series of changes known as gelatinisation causing higher availability of starch to enzymatic breakdown. Bread usually is rapidly digested and fast glucose release and absorption happens after its consumption. It is mainly due to the large amount of rapidly digestible starch content and the small amount of resistant starch (about 2.5 \%) in the baked product after the bread making process (mixing, fermentation and baking). According to the fast glucose response after the consumption, bread is described as a high-GI food playing remarkable role in the development of obesity and diabetes as well as weight gain (Dewettinck et al., 2008, Åkerberg et al., 1998, El, 1999). Based on the observation that bread produces a high GI, efforts have been made to develop bread products that induce lowered glycaemia and reduced demand for insulin. Examples of food factors capable of reducing GI of bread include inclusion of intact cereal kernels, the use of sourdough.
fermentation, and enrichment with viscous fibre and resistant starch as well as the change of baking conditions (long-time, low temperature baking) (Åkerberg et al., 1998). Resistant starches can be used in food products providing good handling and improved texture in the final product. It was shown that next to the appropriate physical (low water holding capacity) and functional properties (good source of dietary fibre) of resistant starches, they can improve or at least not significantly influence the sensory profile of foods. The granular RS was shown to provide better appearance, texture, and mouthfeel than do other, conventional fibre sources. Breads containing RS were determined to have superior quality compared to those made with traditional fibres (Yue and Waring, 1998, Baixauli et al., 2008). Moreover RS can be used in the development of gluten-free bread samples as well without causing significant influence on the organoleptic quality and texture of the product (Korus et al., 2009).

Different commercial sources of RS are known in the market. National Starch provides several types of RS including high- amylose (RS2) starches (Hi-maize products) as well as retrograded starches (RS3) such as Novelose products. Opta Food Ingredients provides Crystalean (RS3), Cerestar provides C*Actistar (RS3) while MGP Ingredients (in Europe it is called Loryma GmbH) manufactures RS4 starch (Fibersym) as phosphate-esterised product. These starches have different functional and nutritional profiles; the investigations of their properties therefore are remarkably important (Sajilata, et al., 2006).

1.2.6 Pasta as a typical low or medium-GI foodstuff

Pasta products are also basic foodstuffs having an important role in the human food consumption. They can be easily prepared, handled, cooked and stored. The most important consumer attribute is the cooking and sensory quality of the pasta which includes the cooking time, water absorption, texture, taste and aroma of the cooked product (Cunin et al., 1995, Grant et al., 1993). Research has shown that sugars are progressively liberated from pasta during digestion, leading to a standard increase in postprandial blood glucose and insulin response (glycaemic index) (Tudorică et al., 2002). Pasta is regarded as a product with low glycaemic index (Björck et al., 2000), however, it would be better to range it among foods with medium GI (GI of pasta is about 50-70). Foods with low glycaemic response can be used in the treatment of obesity, type II diabetes mellitus as well as in weight management. There are several methods to lower glycaemic response of pasta. According to Goñi and Valentín-Gamazo (2003), chickpea flour causes slower glycaemic response from pasta in healthy
volunteers (dried legume seeds generally promote slow and moderate postprandial blood glucose increase (Chillo et al., 2008)). Tudorică et al. (2002) investigated the reduction of postprandial blood glucose level after consumption of pastas containing different types of dietary fibre components (inulin, guar gum, pea fibre). Moreover parallel with the auspicious glycaemic properties, inulin has not shown significant effects neither on the rheology nor the stability of pasta products in more recent research (Brennan et al., 2004). The use of resistant starches in pasta can also improve glucose and lipid metabolism (Nugent, 2005; Liljeberg et al., 1999). Moreover the physical barrier created by the compact structure of the dense protein network resulting from the extrusion process limits the accessibility of starch to amylase and delays in vitro starch hydrolysis (Hoebler et al., 1999, Fardet et al., 1999). Investigating the starch containing pasta, it can be stated that the intrinsic quality attributes are influenced primarily by the properties of the protein and starch fractions and by factors such as origin of the flour and the food production process (Maache-Rezzoug and Allaf, 2005).

The effects of food processing can influence the starch availability and therefore glycaemic properties of pasta. The drying temperatures, hydrothermal process conditions, extrusion conditions, lamination process and cooking have a great impact on the nutritional characteristics of pasta samples (Cunin et al., 1995, Bornet et al., 1990, Zardetto and Dalla Rosa, 2006, Kim et al., 2006, Maache-Rezzoug and Allaf, 2005, Grant et al., 1993, Güler et al., 2002).

Summarizing the introduction part it can be concluded, that the starch based foods have an important role in the human nutrition, therefore the different starch fractions have to be investigated. Resistant starches are dietary fibres having several health benefits indicating their importance in our diet and digestion system. Bread and pasta are two basic foodstuffs with different nutritional characteristics. The effects of bread and pasta on the glycaemic response in the human body vary due to the food processing as well as the type of flour and the ratio of rapidly, slowly digestible and resistant starches. In this thesis studies have been carried out on the digestibility and physical properties of different resistant starches and on their applications and effects in real food materials (bread and pasta) as well as their impact on the GI of developed pasta products.
AIMS OF THE STUDIES

The aims of my PhD studies were the follows:

1. to investigate the physicochemical and digestibility properties of resistant starches of different origin and type and to compare them to native starches
2. to investigate the effects of heat load (dry and moisture heat treatment) on the characteristics of starches
3. to use RS in bread samples and to investigate their effects on the properties (physical, sensory and nutritional) of the bread products
4. to use RS in pasta samples and to investigate their effects on the properties (physical, sensory and nutritional) of the pasta samples
5. to measure the *in vitro* and *in vivo* GI of pasta samples using approved, standard methods with modifications to study the effect of high-amylose RS on the nutritional characteristics of pasta
6. to give help for manufacturers how to use RS in their products prepared by baking, extrusion or cooking.
2 MATERIALS and METHODS

2.1 Native and resistant starches and their properties

Three native starches (maize, wheat and rice), and six resistant starches were studied. The origin and characteristics of the materials are listed in Table 2. The characteristics of native and resistant starches were measured as-is and in their physical mixtures. Wheat starch + Fibersym70 and wheat starch + Hi-maize260 as well as maize starch + Fibersym70 and maize starch + Hi-maize260 mixtures were prepared to investigate the effects of resistant starches in powder blends. The mixtures were made in each case by using the single native starch and the single resistant starch in the ratio of 0, 20, 40, 60, 80, 100 w/w %, respectively. The starches used in the tests are summarized in Table 2.

Table 2 Native and resistant starches used in the studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>Starch character</th>
<th>Product origin</th>
<th>Source(supplier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>Native</td>
<td>Maize</td>
<td>Sigma Aldrich (Budapest, Hungary)</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>Native</td>
<td>Wheat</td>
<td>Sigma Aldrich (Budapest, Hungary)</td>
</tr>
<tr>
<td>Rice starch</td>
<td>Native</td>
<td>Rice</td>
<td>Sigma Aldrich (Budapest, Hungary)</td>
</tr>
<tr>
<td>Hi-maize 260</td>
<td>Resistant (60% total dietary fiber)</td>
<td>Maize</td>
<td>National Starch and Chemical GmbH (Hamburg, Germany)</td>
</tr>
<tr>
<td>Hi-maize 1043</td>
<td>Resistant (54% total dietary fiber)</td>
<td>Maize</td>
<td>National Starch and Chemical GmbH (Hamburg, Germany)</td>
</tr>
<tr>
<td>Novelose 330</td>
<td>Resistant (30% total dietary fiber)</td>
<td>Maize</td>
<td>National Starch and Chemical GmbH (Hamburg, Germany)</td>
</tr>
<tr>
<td>Crystalean</td>
<td>Resistant (30% total dietary fiber)</td>
<td>Maize</td>
<td>Sun Opta Ingredients (Bedford, MA, USA)</td>
</tr>
<tr>
<td>C*Actistar</td>
<td>Resistant (54% total dietary fiber)</td>
<td>Tapioca</td>
<td>Cerestar (Hambourdin, France)</td>
</tr>
<tr>
<td>Fibersym 70</td>
<td>Resistant (70% total dietary fiber)</td>
<td>Wheat</td>
<td>Loryma GmbH (Zwingenberg, Germany)</td>
</tr>
</tbody>
</table>
2.2 Comparison of native and resistant starches

2.2.1 Detection of the rheological properties of starches using rapid visco analyzer (RVA) method

The viscosograms were recorded using a Rapid Visco Analyzer RVA -4SA system (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia). Standard 1 measurement profile (ICC Standard Method No. 162) was used and the viscosity parameters were recorded in cP units (1 cP = 1 mPa s⁻¹). 3.5 g sample was dispersed in 25 mL distilled water, mixed in the RVA aluminium sample bin, and measured. The sample slurry was equilibrated at 50 °C for 1 min (stage 1), heated from 50 to 95 °C in 3 min 42 s (stage 2), maintained at 95 °C for 2.5 min (stage 3), cooled to 50 °C in 3 min 48 s (stage 4), and held at 50 °C for 2 min (stage 5). The paddle speed was 960 rpm for the first 10 s, and 160 rpm for the remainder of the experiment. The viscosogram representing the profile and the RVA parameters is shown in Figure 10.

![Figure 10](image-url) The standard RVA profile and the typical RVA parameters (Application manuals for the RVA, 1998)
Unmodified starch granules are generally insoluble in water below 50 °C. When starch granules are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell to many times their original size. Over a critical temperature range, the starch granules undergo an irreversible process known as gelatinization, which is marked by crystalline melting, loss of birefringence and starch solubilisation.

Early in the pasting test the temperature is below the gel temperature of the starch, and the viscosity is low. When the temperature rises above the gelatinisation temperature, the starch granules begin to swell, and viscosity increases on shearing when these swollen granules have to squeeze past each other. The temperature at the onset of the rise in viscosity is known as the pasting temperature.

When a sufficient number of granules become swollen, a rapid increase in viscosity occurs. Granules swell over a range of temperatures, indicating their heterogeneity of behaviour. This range is reflected in the steepness of the initial rise in viscosity in the pasting curve.

As the temperature increases further, the granules rupture and the more soluble amylose leaches out into solution, followed at a slower rate in some cases by the amylopectin fraction. Granule rupture and subsequent polymer alignment due to the mechanical shear reduces the apparent viscosity of the paste. These combined processes that follow gelatinisation are known as pasting.

**Peak viscosity** occurs at the equilibrium point between swelling and polymer leaching which cause an increase in viscosity, and rupture and polymer alignment which cause it to decrease. It is common to measure the **peak temperature** and **peak time** that occur with the peak viscosity.

During the hold period of the test, the sample is subjected to a period of constant high temperature (usually 95 °C) and mechanical shear stress. This will further disrupt the granules and amylose molecules will generally leach out into solution and undergo alignment.

This period is commonly accompanied by a breakdown in viscosity to a holding strength, hot paste viscosity or **trough**.

As the mixture is subsequently cooled, re-association between starch molecules, especially amylose, occurs to a greater or lesser degree. In sufficient concentration this usually causes the formation of a gel, and viscosity will normally increase to a **final viscosity**. This phase of the pasting curve is commonly referred to as the **setback** region, and involves retrogradation, or re-ordering, of the starch molecules.
Values measured from the pasting profile were determined according to the Applications manual for the RVA (1998) as follows:

1. **the peak viscosity** (maximum paste viscosity achieved in stage 2, the heating stage of the profile);
2. **trough** (minimum paste viscosity achieved after holding at the maximum temperature, stage 3);
3. **final viscosity** (the viscosity at the end of run);
4. **pasting temperature** (the temperature at which starch granules begin to swell and gelatinize due to water uptake and defined as an increase of 25 cP over a period of 20 s);
5. **peak time** (the time at which peak viscosity was recorded);
6. **breakdown** (difference between peak viscosity and trough); and
7. **setback** (difference between final viscosity and trough).

Each sample was analyzed in duplicate.

### 2.2.2 The water absorption (WA) ability of starches

Water absorption (WA) values of starches and their mixtures were determined using the method of Medcalf and Gilles (1965). A suspension of 2 g starch in 30 ml distilled water was agitated for 1 h and centrifuged (3000 g) for 10 min. Then free water was removed from wet starch, drained for 10 min and then wet starch was weighed.

Water absorption of the samples was determined as

\[
\text{Water absorption} = \frac{W_1 - W_2}{W_2} \times 100\%
\]

(1)

Where \( W_1 \) (g) is the weight of wet starch and \( W_2 \) (g) is the weight of dry sample. Each sample was analyzed in duplicates.
2.2.3 The enzymatic digestion of starches

An enzymatic digestion test was performed with 100±5 mg samples (based on dry matter content) according to a simple hydrolyses method (Megazyme, 2004) with some modifications. An enzyme solution of 4 ml containing porcine pancreatic alpha-amylase (110U/mL), EC 3.2.1.1 (Sigma Aldrich, Budapest, Hungary) and amyloglycosidase (3U/ml), EC 3.2.1.3 (Sigma Aldrich, Budapest, Hungary) from Aspergillus niger was used during the procedure at pH=6.9 (0.05 M sodium potassium phosphate buffer). After an incubation of 16h at 37 °C the amount of liberated glucose was measured using the GOPOD (glucose-oxidase EC 1.1.3.4; peroxidase, EC 1.11.1.7) enzymatic kit (Fábió Co. Ltd, Budapest, Hungary). Test method was calibrated with the given standard glucose solution (5.55 mmol L⁻¹). Samples were measured in triplicates. Results were given as liberated glucose mg×mL⁻¹. Each sample was analyzed in triplicates and results were expressed as mean±SEM (standard error of mean). The data were analyzed with T-tests using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005).

2.3 The effects of heat treatment on starches

2.3.1 Dry heat treatment of native and resistant starches

The dry starch samples were heat treated in an air-oven (T-5028 Heraeus, Heraeus Holding GmbH, Germany) according to a 3^2 factor design experiment. The factors were the temperature (80 °C, 100 °C and 120 °C) and the time of heat load (10, 20, 30 min). The properties of starches were detected with RVA, WA and enzymatic digestion methods as described above.

2.3.2 Moisture heat treatment (cooking) of native and resistant starches

Starches (5g) were cooked for 10 min in 50 mL of distilled water. After cooking the samples were cooled down to room temperature and were digested in the enzymatic method described above.
2.3.3 Selection of starches for bread and pasta production

After the investigation of starches and the effects of heat load on starch properties, three resistant starches were chosen to use them in products such as bread and pasta. These were Hi-maize 260, Hi-maize 1043 and Fibersym 70 starches.

2.4 Bread baking with resistant starch addition

White breads were made from commercial bread flour (BL-80, ash content 0.80%, Ferencvárosi Mills, Budapest, Hungary) and from the three selected resistant starch products: A starch (Hi-maize 260, National Starch and Chemical GmbH, Hamburg, Germany), B starch (Fibersym 70, Loryma GmbH, Zwingenberg, Germany) and C starch (Hi-maize 1043, National Starch and Chemical GmbH, Hamburg, Germany). Resistant starch ingredients were incorporated into recipes by replacing wheat flour at 20 (w/w) % level. The lack of gluten caused by the RS addition (in all RS added products) was compensated by adding the proper amount of wheat gluten (Sigma Aldrich, Budapest, Hungary) into the bread dough. According to the supplier the protein content of the bread flour was 12 %. It can be calculated that due to the RS addition 2.4 % gluten had to be inserted into the samples. Control bread without resistant starch addition was also prepared. For shortening the titles and descriptions of the samples a code was used instead of the names of resistant starches. Accordingly A starch was Hi-maize 260, B was Fibersym 70 and C was Hi-maize 1043. The breads were baked using conventional baking conditions (30 min, 230 °C) and were made as follows. A suspension of yeast (3.5%) in water was mixed with the wheat flour and the resistant starches and 2% salt was also added. The ingredients were formed into dough that was divided into two loaves. The loaves were put into baking pans and proofed for 2 h (30 °C) and then baked in an air oven at 230 °C until yellow-brownish colour. The proofing and baking were carried out in uncovered pans. After baking the products were cooled down to room temperature and their volumes, weights as well as sensorial properties were investigated. The RS content as well as the enzymatic digestibility were measured on the next day.
2.5 Comparison of different bread products

2.5.1 Rheological properties of bread samples measured by RVA method

RVA curves were determined using the bread raw materials before baking. The viscograms were recorded using a Rapid Visco Analyzer RVA -4SA (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia). Standard 1 measurement profile (ICC Standard Method No. 162) was used and the viscosity parameters were recorded in cP units (1 cP = 1 mPa s⁻¹). 3.5g sample (particle size <0.5 mm) were dispersed in 25 mL distilled water, mixed in the RVA aluminium sample bin, and measured. The method has been described in section 2.2.1. Samples were measured in duplicates.

2.5.2 Physical and sensorial properties of bread products

After cooling down to room temperature the weight of the products was measured using a simple scale while the volume of the bread samples was measured according to the standard methodology using mustard-seed (Hungarian Standard, 20501/3-82, 1982). Bread samples were submitted to a panel of 10 independent tasters for the estimation of shape, crumb and crust properties as well as smell and taste on a 1-5 point scale (according to the Hungarian Standard 20501/2-82, 1982) with some modifications. In our case the shape was not included into the tests; the bread samples were namely prepared in baking pans, therefore 5 point was added in all cases. The crumb has to be typical for the product, uniformly smooth, not sticky and dense and should not contain any strange materials. The crust has to be typical for the product, uniformly smooth, glossy and the colour might be from golden yellow to mild brown. The smell should be typical for bread without any inconvenient effects (sourish, rancid or strange smell). The taste should also be typical for bread without any inconvenient effects (sourish, rancid or off-flavour taste). The products can be ranged based on the weighted total score (expressed as: 0.6×score of shape + 0.6×score of crust + 1.4×score of crumb + 0.4×score of smell + 1.0×score of taste) into five categories (Table 3).

Table 3 Bread quality categories according to the weighted total score

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.6-20.0</td>
<td>excellent</td>
</tr>
<tr>
<td>15.2-17.5</td>
<td>good</td>
</tr>
<tr>
<td>13.2-15.1</td>
<td>moderate</td>
</tr>
<tr>
<td>11.2-13.1</td>
<td>accepted</td>
</tr>
<tr>
<td>under 11.2</td>
<td>not acceptable</td>
</tr>
</tbody>
</table>
2.5.3 Enzymatic digestibility and RS content of bread products

The procedure described by Tudorică et al. (2002) was used with some modification. The enzymatic digestion test was performed with 2.00± 0.10 g (concerning to dry material content) samples (raw bread dough samples and baked products) mixed in 8 ml porcine pancreatic alpha-amylase (110U/ml), EC 3.2.1.1 (Sigma Aldrich, Budapest, Hungary), at pH=6.9. Before enzymatic digestion the bread samples (mainly crumb) were cut into small pieces (∼1 cm³) to be able to get mixed in the enzyme solution. Samples were incubated at 37 °C and sampling was carried out after 0, 30, 60, 90, 120, 180 min. The amount of liberated glucose (mg glucose/g sample) was measured using the GOPOD (glucose-oxidase EC 1.1.3.4; peroxidase, EC 1.11.1.7) enzymatic kit (Fábió Co. Ltd, Budapest, Hungary). Test method was calibrated with the given standard glucose solution (5.55 mmol L⁻¹). Samples were measured in triplicates. The kinetic curves were evaluated using the GraphPad Prism 4 for Windows software (GraphPad Software, Inc, San Diego, USA, 2003).

The resistant starch content of the samples was determined according to the Megazyme method (2004). The method is applicable to samples containing more than 2 w/w %RS. With such samples, standard errors of ± 5 % are achieved routinely. Higher errors are obtained for samples with RS contents < 2 w/w %. Samples were measured in triplicates.

The data were analyzed statistically by ANOVA followed by T-tests for independent samples and Dunnett’s post hoc test using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005). Results from the tests were obtained as means±SEM (standard error of the mean). Significance level was p<0.05.

2.6 Pasta preparation with resistant starch addition

Two wheat flour types used in the pasta processing were from Triticum aestivum (common wheat) and Triticum durum (hard wheat) species. The two species present different genetic features as well as morphological properties. The products obtained from each variety differ significantly. Common wheat flour is white and dusty while durum semolina has rough granules and pointed (sharp), yellow particles (Brovedani and Tyfield, 2000). Accordingly the products from the two different varieties should have different properties. The T. aestivum
wheat flour and the *T. durum* wheat semolina used in the pastas are specified in *Codex Alimentarius Hungaricus, 2007*.

*T. aestivum* wheat pasta was made using commercial *T. aestivum* wheat flour (TL-80, ash content 0.80 %, Júlia Mills, Kunszállás, Hungary), whole egg powder, water and the different added resistant starches: A starch (Hi-maize 260, National Starch and Chemical GmbH, Hamburg, Germany), B starch (Fibersym 70, Loryma GmbH, Zwingenberg, Germany), or C starch (Hi-maize 1043, National Starch and Chemical GmbH, Hamburg, Germany). *T. durum* wheat pasta was made in the same way using commercial *T. durum* wheat semolina (TD-50, ash content: 0.50 %, Júlia Mills, Kunszállás, Hungary) without egg powder additive.

Added RS components were incorporated into recipes by replacing wheat flour at 10 and 20 (w/w) % levels. Control pasta with no RS addition was also prepared. Whole egg powder was added at 2.1 (w/w) % to each *T. aestivum* sample. The moisture content of the flour, starch and egg powder mixtures was adjusted during intensive mixing of dough (30 min), taking into account the different water absorption levels (measurements described by Medcalf and Gilles, 1965) of the RS-s. We aimed to produce optimal appearance and sensory properties of the dough prior to extrusion. The moisture content of the pasta mixtures was about 22-26 % before extrusion according to the type and amount of RS-s. The pasta-doughs were mixed and extruded using a WLS LOSER PRESSQUICK (Loser GmbH, Karlsruhe, Germany) pilot-plant installation. After a mixing of 20 min the pastas were extruded under standard conditions (120 bar, 40 °C) and cut into the form of short cut pasta (2-3 cm approximately) (**Figure 11**).
In large scale pasta production, vacuum mixing is carried out performing two important functions. First, oxygen is removed, reducing oxidation of yellow pigments and giving pasta a more intense color. Second, vacuum prevents the formation of air bubbles. If air bubbles are present, the dried pasta is less bright, and has less mechanical strength (Smith and Hui, 2004). In my case, in WLS LOSER PRESSQUICK pilot-plant installation, the vacuum mixing was not possible, however, the quality of the products was acceptable. The products were encoded according to the type of flour (*T. aestivum* or *T. durum*), the RS used as a flour replacer (Hi-maize 260=A starch, Fibersym70=B starch, Hi-maize1043= C starch) as well as the amount of resistant starches (10, 20 %). The codes of the pastas are summarized in Table 4.

**Table 4 Encoding of the pasta samples**

<table>
<thead>
<tr>
<th>Pasta</th>
<th>Flour</th>
<th>Resistant starch</th>
<th>Amount of RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>T.aestivum</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>AA10</td>
<td>T.aestivum</td>
<td>Hi-maize 260</td>
<td>10</td>
</tr>
<tr>
<td>AA20</td>
<td>T.aestivum</td>
<td>Hi-maize 260</td>
<td>20</td>
</tr>
<tr>
<td>AB10</td>
<td>T.aestivum</td>
<td>Fibersym 70</td>
<td>10</td>
</tr>
<tr>
<td>AB20</td>
<td>T.aestivum</td>
<td>Fibersym 70</td>
<td>20</td>
</tr>
<tr>
<td>AC10</td>
<td>T.aestivum</td>
<td>Hi-maize 1043</td>
<td>10</td>
</tr>
<tr>
<td>AC20</td>
<td>T.aestivum</td>
<td>Hi-maize 1043</td>
<td>20</td>
</tr>
<tr>
<td>D control</td>
<td>T.durum</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>DA10</td>
<td>T.durum</td>
<td>Hi-maize 260</td>
<td>10</td>
</tr>
<tr>
<td>DA20</td>
<td>T.durum</td>
<td>Hi-maize 260</td>
<td>20</td>
</tr>
<tr>
<td>DB10</td>
<td>T.durum</td>
<td>Fibersym 70</td>
<td>10</td>
</tr>
<tr>
<td>DB20</td>
<td>T.durum</td>
<td>Fibersym 70</td>
<td>20</td>
</tr>
<tr>
<td>DC10</td>
<td>T.durum</td>
<td>Hi-maize 1043</td>
<td>10</td>
</tr>
<tr>
<td>DC20</td>
<td>T.durum</td>
<td>Hi-maize 1043</td>
<td>20</td>
</tr>
</tbody>
</table>

Samples were dried to a final moisture content of 10 % wet basis in an air-drying room at 35-40 °C in a 24-h drying cycle and their properties were evaluated within two weeks.

**2.7 Comparison of different pasta products**

**2.7.1 Rheological properties of pasta products measured by RVA method**

RVA curves were determined using the pasta raw mixtures. The viscograms were recorded using a Rapid Visco Analyzer RVA -4SA (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia). Standard 1 measurement profile (ICC Standard Method No. 162) was used and the viscosity parameters were recorded in cP units (1 cP = 1 mPa s⁻¹).
Sample of 3.5 g (particle size <0.5 mm) were dispersed in 25 mL distilled water, mixed in the RVA aluminium sample bin, and measured. The method has been described in section 2.2.1. Samples were measured in duplicates.

2.7.2 Comparison of cooking properties of different pasta products

Pasta (25g) was cooked for 5 min (optimum cooking time) in 250 mL of boiling distilled water. Optimum cooking time (the time that is necessary to obtain complete gelatinization of starch) was defined as the time required for the white, opaque, core of the strand to disappear. The time was determined by removing a strand from the water at 30 s intervals and cutting it into two pieces. After cooking, the samples were rinsed with distilled water in a Buchner funnel and allowed to drain for 2 min according to the method described by Grant et al. (1993). Pasta quality is influenced by several physical, chemical, textural and nutritional characteristics. Cooking time, swelling index, water absorption and cooking loss as well as the texture of the cooked products and consumer values, such as stickiness, aroma and taste, are the most important characteristics influencing the sensory quality of pasta. Accordingly, after cooking and draining, samples were analyzed for swelling index, water absorption and dry matter content (Tudorică et al., 2002) as well as cooking loss using the method described by Grant et al. (1993). Measurements were repeated four times.

**Swelling index** of cooked pasta was evaluated by drying pasta samples to constant weight at 105 °C for 4 hours, expressed as

\[
\frac{W_1 - W_2}{W_2} \quad (2)
\]

Where \(W_1\) (g) is the weight of cooked product and \(W_2\) (g) is the weight after drying.

**Water absorption** of drained pasta was determined as

\[
\frac{W_1 - W_3}{W_3} \times 100\% \quad (3)
\]

Where \(W_3\) (g) is the weight of raw pasta.

**Dry matter** of cooked pasta was determined after drying the samples to constant weight at 105 °C, expressed as

\[
\frac{W_2}{W_1} \times 100\% \quad (4)
\]
**Cooking loss** was evaluated after the combining of the cooking and rinse waters in a weighed beaker and evaporating them to dryness in an air-oven at 110 °C. The residue was weighed and reported as a percentage of the original pasta sample.

The data were analyzed statistically by ANOVA followed by T-tests for independent samples and Dunnett’s post hoc test using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005). Results from the tests were obtained as means±SEM (standard error of the mean). Significance level was p<0.05.

### 2.7.3 Sensory properties of different pasta products

Optimally cooked pasta samples were submitted to a panel of 10 independent tasters for estimation of appearance, smell, taste and textural quality on a 1-5 point scale (according to the Hungarian Standard 20500/3-85, 1986). The appearance of pasta includes the investigation of shape, colour differences and the amount of cracked pieces. The smell should be typical for pasta without any strange odour (sourish, rancid or strange smell). The taste should also be typical for pasta without any strange flavour (sourish, rancid or off-flavour taste). The textural quality includes the stickiness, firmness and elasticity of cooked pastas. The products can be classified based on the weighted total score (expressed as: 1.1×score of appearance + 0.7×score of smell + 0.9×score of taste + 1.3×score of texture) into three categories. Pastas having a total score of 16 to 20 are in the first class, products with total score of 11.2 to 15.9 are in the second class and the samples under 11.2 can not be acceptable.

### 2.7.4 Enzymatic digestion and resistant starch content of pasta products (pasta raw mixtures, samples after extrusion and drying and cooked pastas)

The procedure described by Tudorică et al. (2002) was used with some modification. The enzymatic digestion test was performed with 5.00±0.10 g of cooked pasta samples or 2.00 ± 0.10 g of raw mixtures and extruded samples (concerning to dry material content) mixed in 20 mL porcine pancreatic alpha-amylase (110U/mL), EC 3.2.1.1 (Sigma Aldrich, Budapest, Hungary), pH=6.9 (0.05 M sodium potassium phosphate buffer). Before enzymatic digestion the cooked pastas were rubbed manually in a mortar to be able to mix them with the enzyme
solution. Samples were incubated at 37 °C and sampling was carried out after 0, 30, 60, 90, 120, 180 min. The amount of liberated glucose was measured using the GOPOD (glucose-oxidase EC 1.1.3.4; peroxidase, EC 1.11.1.7) enzymatic kit (Fábió Co. Ltd, Budapest, Hungary). Test method was calibrated with the given standard glucose solution (5.55 mmol×L⁻¹). Samples were measured in triplicates.

The kinetic curves were evaluated using the GraphPad Prism 4 for Windows software (GraphPad Software, Inc, San Diego, USA, 2003).

The resistant starch content of the samples was determined according to the Megazyme method (2004). Samples were measured in triplicates.

The data were analyzed statistically by ANOVA followed by T-tests for independent samples and Dunnett’s post hoc test using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005). Results from the tests were obtained as means±SEM (standard error of the mean). Significance level was p<0.05.

2.7.5 Pasta selected for in vitro and in vivo GI tests

After the comparison of the properties of different RS enriched pasta products, the sample with the best sensorial and digestibility properties (AA20) and the proper control pasta were selected for further in vitro as well as in vivo GI tests.

2.8 In vitro GI test of the chosen RS enriched pasta

The methods of Holm and Björck (1992) as well as Germaine et al. (2008) were used with some modifications. Pastas were cooked according to the cooking procedure described in section above. After rinsing and cooling to room temperature the samples were minced through a 2 mm plate and portions of the minced food containing 1g available carbohydrate were weighed into centrifuge tubes. Samples containing 1g available carbohydrate from the commercial white bread used as reference were also weighed into centrifuge tubes. The samples were mixed with 6 mL pepsin (E.C. 3.4.23.1, Sigma Aldrich, Budapest, Hungary) solution (100U enzyme in 6 mL 0.05 M sodium potassium phosphate buffer containing 0.4 g
NaCl/L, adjusted to pH=1.5 with 2M HCl) and incubated at 37 °C for 30 min with stirring twice during the incubation. After this phase the pH was adjusted to 6.9 with 2M NaOH and 5 mL porcine pancreatic alpha-amylase (110U/mL in 0.05 M sodium potassium phosphate buffer containing 0.3 g CaCl₂/L), EC 3.2.1.1 (Sigma Aldrich, Budapest, Hungary) was added to each sample. The solutions were diluted to 30 mL and incubated at 37 °C for 3 hours. Sampling was carried out after 0, 30, 60, 90, 120, 180 min. The amount of liberated glucose was measured using the GOPOD (glucose-oxidase EC 1.1.3.4; peroxidase, EC 1.11.1.7) enzymatic kit (Fábió Co. Ltd, Budapest, Hungary). Test method was calibrated with the given standard glucose solution (5.55 mmol L⁻¹). Samples were measured in triplicates.

The kinetic curves were evaluated using the GraphPad Prism 4 for Windows software (GraphPad Software, Inc, San Diego, USA, 2003). Results were compared by T-Tests using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005). Results from the tests were obtained as means±SEM (standard error of the mean). Significance level was p<0.05.

2.9 In vivo GI test of the chosen RS enriched pasta

The GI measurement of the selected pasta samples (T. aestivum pasta with 20 % Hi-maize260 and T. aestivum control) was carried out at the Department of Human Biology, University of Maastricht (the Netherlands) following the standard methodology observing strict instructions (Brouns et al., 2005) with 10 volunteers using bread as reference food.

The food was served for breakfast and had to be consumed in 15 minutes. Blood sampling was carried out just before eating (at 0 min) and after eating at 15, 30, 45, 60, 90 and 120 min. The blood samples were centrifuged (3000 rpm, 4 °C, 10 min) cooled down in liquid nitrogen and stored at -80 °C until analysis. The glucose content of the blood samples was measured using the hexokinase method (Glucose HK 125 kit, ABX diagnostics, Montpellier, France). The kinetic curves were evaluated according to the standard methodology for the calculation of the glycaemic index. Results were compared by T-tests using Statistica 7.1 (StatSoft Inc, Tulsa, USA, 2005). Results from the tests were obtained as means±SEM (standard error of the mean). Significance level was p<0.05.
3 RESULTS and DISCUSSION

3.1 Comparison of native and resistant starches

Native wheat, maize and rice starches were compared to resistant starches according to their rheological properties, water absorption ability and enzymatic digestibility. Rheological properties and water absorption ability play important role in the product development with RS-s as the characters of cereal based products like pasta, bread as well as extrudates are mostly influenced by the water content and the texture of these foodstuffs. Enzymatic digestion tests were also carried out to describe the differences among the starches according to their resistance and to prove the unavailability of RS-s to amylolytic attack.

3.1.1 Detection of the rheological properties of starches and their stochiometric mixtures using rapid visco analyzer (RVA) method

The RVA curves of native and resistant starches are shown in Figure 12. Native starches owned different RVA curves due to their different plant origin, diverse structure of the starch crystals as well as different water binding capacity. The RVA method has been earlier confirmed to be able to differentiate the starches according to their origin and structure (Jane et al., 1999).

![RVA Viscograms of Native Starches and Resistant Starches](image)

**Figure 12** RVA viscosgrams of native starches and resistant starches. **a**: maize starch; **b**: wheat starch; **c**: rice starch; **d**: resistant starches (magnified in small frame)
The characteristic RVA parameters of the different starches—summarized in Table 5—show high variability according to the type and origin of the sample. Among the native starches all of the seven parameters seemed to be sensitive enough to follow up the changes during the RVA procedure.

**Table 5** The RVA parameters of native and resistant starches

<table>
<thead>
<tr>
<th>Starch</th>
<th>PV (cP)</th>
<th>TR (cP)</th>
<th>BD (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
<th>PT (min)</th>
<th>PTp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>3041.5</td>
<td>2120</td>
<td>921.5</td>
<td>3207</td>
<td>1087</td>
<td>5.13</td>
<td>76.33</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>3423.5</td>
<td>2933</td>
<td>490.5</td>
<td>4160</td>
<td>1227</td>
<td>6.99</td>
<td>83.5</td>
</tr>
<tr>
<td>Rice starch</td>
<td>2487</td>
<td>2158</td>
<td>329.5</td>
<td>3334</td>
<td>1176</td>
<td>6.63</td>
<td>80.68</td>
</tr>
<tr>
<td>Hi-maize 260</td>
<td>19</td>
<td>2.5</td>
<td>16.5</td>
<td>13</td>
<td>10.5</td>
<td>5.93</td>
<td>-</td>
</tr>
<tr>
<td>Hi-maize 1043</td>
<td>24</td>
<td>1</td>
<td>23</td>
<td>12</td>
<td>11</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>Novelose 330</td>
<td>33</td>
<td>18</td>
<td>15</td>
<td>22</td>
<td>4</td>
<td>4.99</td>
<td>-</td>
</tr>
<tr>
<td>Crystalean</td>
<td>45</td>
<td>27</td>
<td>18</td>
<td>38</td>
<td>11</td>
<td>6.06</td>
<td>-</td>
</tr>
<tr>
<td>C*Actistar</td>
<td>23</td>
<td>6</td>
<td>17</td>
<td>5</td>
<td>-1</td>
<td>5.79</td>
<td>-</td>
</tr>
<tr>
<td>Fibersym 70</td>
<td>18</td>
<td>3</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>3.73</td>
<td>-</td>
</tr>
</tbody>
</table>

*PV*: peak viscosity; *TR*: trough; *BD*: breakdown; *FV*: final viscosity; *SB*: setback; *PT*: peak time; *PTp*: pasting temperature

Values represent means of duplicates

RS products (see magnified part of Figure 12 and the parameters in Table 5) did not show any significant viscosity changes during the applied STD1 profile. The recorded RVA parameters have confirmed the unchanged viscosity of these starches during the heat treatment (gelatinization) procedure. Gelatinization properties of starches generally depend on the type, granular structure, botanical origin as well as amylose/amylopectin ratio of the sample (Sajilata et al., 2006). The RS2-type and RS3-type starches have high amylose content leading to lower gelatinization affinity using the standard RVA procedure (Jane et al., 1999).

Probably due to its chemical modification, Fibersym 70, the RS4-type phosphate-ester starch is not able to gelatinize in this method either and therefore shows only insignificant viscosity parameters. Xie and Liu (2004) proved that the chemically modified citrate starches have small affinity to gelatinize and during cooking do not show any changes. Phosphate-ester starches might be also resistant against cooking and heat treatment in the RVA system. Functional properties like viscosity and the pasting properties of RS components are important characteristics and must be considered in food applications.
The functional effects of RS products in mixtures are summarized in Figure 13, 14, 15 and 16. The series of RVA curves of wheat starch+Fibersym 70 and wheat starch+Hi-maize 260 as well as maize starch+Fibersym 70 and maize starch+Hi-maize 260 indicate that the effects of RS components on the functional properties are strongly significant; the changes of the parameters however are not stoichiometric.

In the wheat starch mixtures both resistant starch components had similarly significant decreasing effect on the gelatinization properties and typical viscosity values.

Figure 13 RVA curves of mixtures: wheat starch + Fibersym 70

Figure 14 RVA curves of mixtures: wheat starch + Hi-maize 260
Also in the maize starch mixtures both resistant starch components had similarly significant decreasing effect on the gelatinization properties and typical viscosity values.

![Figure 15 RVA curves of mixtures: maize starch + Fibersym 70](image1)

![Figure 16 RVA curves of mixtures: maize starch + Hi-maize 260](image2)

Among the parameters there were five (peak viscosity, trough, breakdown, setback and final viscosity) which were sensitive enough to follow up the effects of resistant starches in the mixtures. The viscosity values were reduced approximately by 40–60% by adding 20% RS into the mixtures additionally the viscosity reducing effect of the starches was more
increasing with the amount of RS (see Table 6). The RVA curves of mixtures with 80% RS content were only slightly different from the pure RS-s. Although the characters as well as the running of curves were not changed notable by the RS addition. Results also attract the attention that as the resistant starches did not show gelatinization properties, they might have only dilution effect during the RVA cooking leading to changed texture in food applications as well.

Table 6 Changes of RVA parameters in maize and wheat starch mixtures depending on the resistant starch content

<table>
<thead>
<tr>
<th>Mixture</th>
<th>PV (cP)</th>
<th>TR (cP)</th>
<th>BD (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
<th>PT (min)</th>
<th>PTp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch+Hi-maize 260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% RS2</td>
<td>3041.5</td>
<td>2120</td>
<td>921.5</td>
<td>3207</td>
<td>1087</td>
<td>5.1</td>
<td>76.3</td>
</tr>
<tr>
<td>20% RS2</td>
<td>1763.3</td>
<td>1310.3</td>
<td>453</td>
<td>1859</td>
<td>548.6</td>
<td>5.4</td>
<td>82.6</td>
</tr>
<tr>
<td>40% RS2</td>
<td>895</td>
<td>740.5</td>
<td>154.5</td>
<td>936.5</td>
<td>196</td>
<td>5.5</td>
<td>89.2</td>
</tr>
<tr>
<td>60% RS2</td>
<td>262.5</td>
<td>221.5</td>
<td>41</td>
<td>270</td>
<td>48.5</td>
<td>5.6</td>
<td>93.6</td>
</tr>
<tr>
<td>80% RS2</td>
<td>43.5</td>
<td>31.5</td>
<td>12</td>
<td>41</td>
<td>9.5</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>100% RS2</td>
<td>19</td>
<td>2.5</td>
<td>7</td>
<td>13</td>
<td>10</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Maize starch+Fibersym 70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% RS4</td>
<td>3041.5</td>
<td>2120</td>
<td>921.5</td>
<td>3207</td>
<td>1087</td>
<td>5.1</td>
<td>76.3</td>
</tr>
<tr>
<td>20% RS4</td>
<td>1816</td>
<td>1373.3</td>
<td>442.6</td>
<td>1809.3</td>
<td>436</td>
<td>5.4</td>
<td>83.3</td>
</tr>
<tr>
<td>40% RS4</td>
<td>923</td>
<td>719.3</td>
<td>206.6</td>
<td>857.3</td>
<td>138</td>
<td>5.5</td>
<td>89.1</td>
</tr>
<tr>
<td>60% RS4</td>
<td>336.3</td>
<td>234.6</td>
<td>101.6</td>
<td>268</td>
<td>33.3</td>
<td>5.8</td>
<td>93.9</td>
</tr>
<tr>
<td>80% RS4</td>
<td>47.5</td>
<td>21.5</td>
<td>26</td>
<td>29</td>
<td>7.5</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>100% RS4</td>
<td>18</td>
<td>3</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Wheat starch+Hi-maize 260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% RS2</td>
<td>3423.5</td>
<td>2933</td>
<td>490.5</td>
<td>4160</td>
<td>1227</td>
<td>6.9</td>
<td>83.5</td>
</tr>
<tr>
<td>20% RS2</td>
<td>1546.5</td>
<td>1178.5</td>
<td>368</td>
<td>2100</td>
<td>921.5</td>
<td>6.1</td>
<td>90.7</td>
</tr>
<tr>
<td>40% RS2</td>
<td>568.5</td>
<td>417.5</td>
<td>151</td>
<td>1049</td>
<td>631.5</td>
<td>5.4</td>
<td>92.4</td>
</tr>
<tr>
<td>60% RS2</td>
<td>134.5</td>
<td>98.5</td>
<td>36</td>
<td>212.5</td>
<td>114</td>
<td>6.7</td>
<td>94.3</td>
</tr>
<tr>
<td>80% RS2</td>
<td>26.5</td>
<td>16</td>
<td>10.5</td>
<td>27</td>
<td>11</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>100% RS2</td>
<td>19</td>
<td>2.5</td>
<td>7</td>
<td>13</td>
<td>10</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Wheat starch+Fibersym 70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% RS4</td>
<td>3423.5</td>
<td>2933</td>
<td>490.5</td>
<td>4160</td>
<td>1227</td>
<td>6.9</td>
<td>83.5</td>
</tr>
<tr>
<td>20% RS4</td>
<td>1649.5</td>
<td>1340</td>
<td>309.5</td>
<td>2024</td>
<td>778.5</td>
<td>6.5</td>
<td>89.2</td>
</tr>
<tr>
<td>40% RS4</td>
<td>634.5</td>
<td>452</td>
<td>182.5</td>
<td>1216.5</td>
<td>670</td>
<td>5.5</td>
<td>93.3</td>
</tr>
<tr>
<td>60% RS4</td>
<td>117.5</td>
<td>102.5</td>
<td>15</td>
<td>339.5</td>
<td>237</td>
<td>5.8</td>
<td>78.9</td>
</tr>
<tr>
<td>80% RS4</td>
<td>33.5</td>
<td>21</td>
<td>12.5</td>
<td>45.5</td>
<td>24.5</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>100% RS4</td>
<td>18</td>
<td>3</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>3.7</td>
<td></td>
</tr>
</tbody>
</table>

PV: peak viscosity; TR: trough; BD: breakdown; FV: final viscosity; SB: setback; PT: peak time; PTp: pasting temperature
Values represent means of duplicates
3.1.2 The water absorption properties of starches

The water absorption (WA) capacity of starches can influence the status and mobility of water in different applications. Figure 17 shows the WA values of native starches and resistant samples.

![Figure 17 Water absorption of the investigated starches](image)

The native starches have shown slight variation in the WA values between 77.61% and 83.79%. Significantly (p<0.05) higher WA was observed for RS2-type and RS3-type starches while for RS4, a decreased WA was measured compared to the native components. The extremely high WA values detected for RS3 products indicating the elevated water binding surface of retrograded starches. Water absorption values were also determined in native and resistant starch mixtures (Figure 18) where a linear enhancing effect of Hi-maize 260 and a linear decreasing effect of Fibersym 70 were confirmed.
Figure 18 Changes in water binding capacity in wheat and maize starch mixtures

The WA of resistant starches and their effects in mixtures have to be considered and calculated in the product development to be able to predict the water needed for the proper texture of the foodstuffs such as pasta and bread or other starch-based products.
3.1.3 The enzymatic digestion of starches

The digestibility characterized by the liberated glucose from different native and resistant starches is summarized in Figure 19. Native starch samples have a very different character in terms of amylolytic digestibility. Wheat starch has been highly degradable while maize and rice starch have shown a significantly lower availability. This phenomenon might occur due to the different structure and morphology of the starch granules present in the samples.

![Digestibility of native and resistant starches](image)

**Figure 19** Digestibility of native and resistant starches

The RS2-type (high amylose content) and the RS4-type (chemically modified phosphate-ester) products have shown strong resistance against amylase attack, the amount of liberated glucose has been reduced by 70–80% compared to their native versions (maize and wheat starches). The three different RS3-type products represented a less resistant character; their degradability was lower only by 35–55%, compared to the native maize and wheat starches. Investigating the starches it was obvious that chemical modification (Fibersym 70) had a significant effect on enzymatic digestibility of wheat starch.

In food applications, native and resistant starch components can be used in different ratios, so it is important to determine whether enzymatic digestibility changes linearly in stoichiometric mixtures or there is any synergistic effect in mixtures. **Figure 20** summarizes the digestion
ratio of wheat and maize starch mixtures with the Hi-maize 260 and Fibersym 70 components in different levels. The digestion ratio of 100% was represented first by the pure wheat starch and in the other case by the pure maize starch.

Both in wheat and maize starch mixtures, linear relationships were observed between enzymatic digestibility and the amount of resistant starch components. The fitted lines confirmed a strong linearity. In these studies the effects of RS on starch mixtures were proved and statistically confirmed.
Summarizing the results with pure resistant starches it can be concluded that

1, resistant starches are not able to gelatinize in the applied RVA procedure, consequently the viscosity parameters of the mixtures prepared by RS addition decrease significantly with the amount of RS (diluting effect). The texture of the products prepared with RS-s might be notably influenced by the rheological properties of RS.

2, the water absorption (WA) values of resistant starches are very variable according to the type and origin of RS; the WA values have to be calculated and considered when a product is to be developed.

3, the enzymatic digestibility of different RS-s varies according to their origin and type (RS2, RS3, and RS4). Lower resistance can be observed in the case of RS3 starches. The Hi-maize type starches and the chemically modified Fibersym 70 (phosphate-ester starch) seem to be more resistant against the amylolytic attack.

The properties of starches are greatly influenced by the processes of the food industry; the changes of the characters therefore have to be taken into account and investigated. The results of the applied heat treatments on starches are summarized in the next section.
3.2 The effects of heat treatment on starches

3.2.1 Dry heat treatment of native and resistant starches

Evaluating the tests it could be concluded that the heat load at 80 °C did not cause significant changes in the properties of the starches, while after the heat load at 100 °C and 120 °C similar effects could be observed. The time of the treatment was a significant factor (p<0.05) as well as the interaction between the temperature and the time. The temperature itself however was not a statistically important factor. The results at 100 °C are hereby deeply discussed.

3.2.1.1 RVA changes of the starches

The effects of heat treatment on the rheological properties of starches have been investigated in several studies (Muir et al., 1995, van den Elinde et al., 2004, Barron et al., 2001). Generally the gelatinization affinity and the strength of gel are influenced by the applied food processing (cooking, baking, extrusion etc.) In our studies the running of RVA curves and the changes in the RVA parameters were recorded and evaluated. Figure 21 represents the viscosity profile of wheat starch without treatment and after the heat load at 100 °C for 10, 20 and 30 minutes. The heat load caused the increase by 10 to 15 % of the typical viscosity parameters (peak viscosity, trough, breakdown, final viscosity and setback) in the case of the three native (wheat, maize and rice) starches (Table 7 represents the parameters of wheat starch, the RVA curves and parameters of maize and rice starches are shown in the supplement representing similar changes due to heat treatment).

![Figure 21](image-url)
Table 7 The RVA parameters of wheat starch

<table>
<thead>
<tr>
<th>Wheat</th>
<th>PV (cP)</th>
<th>TR (cP)</th>
<th>BD (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
<th>PT (min)</th>
<th>PTp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>3235.5</td>
<td>2816.5</td>
<td>419.0</td>
<td>3944.0</td>
<td>1127.5</td>
<td>7.0</td>
<td>83.9</td>
</tr>
<tr>
<td>10min</td>
<td>3528.5</td>
<td>3038.0</td>
<td>490.5</td>
<td>4330.0</td>
<td>1292.0</td>
<td>7.0</td>
<td>84.4</td>
</tr>
<tr>
<td>20min</td>
<td>3664.5</td>
<td>3152.0</td>
<td>512.5</td>
<td>4513.0</td>
<td>1361.0</td>
<td>7.0</td>
<td>82.3</td>
</tr>
<tr>
<td>30min</td>
<td>3553.0</td>
<td>3020.5</td>
<td>532.5</td>
<td>4344.5</td>
<td>1324.0</td>
<td>7.0</td>
<td>83.9</td>
</tr>
</tbody>
</table>

PV: peak viscosity; TR: trough; BD: breakdown; FV: final viscosity; SB: setback; PT: peak time; PTp: pasting temperature
Values represent means of duplicates

The heat load induced a statistically significant (p<0.05) rise in peak viscosity, trough, breakdown, final viscosity, and setback. The peak time and the pasting temperature did not seem to be sensitive enough to describe the effects of the treatment on starches.

According to the results it can be concluded that the studied native starches showed sensitivity on the heat load in dry form; moreover, their rheological properties might be variable depending on the food processing used in the preparation of foodstuffs.

In the case of resistant starches the RVA profile was not affected by the heat treatment. The gelatinization affinity of these starches did not increase due to the heat load. After the measurements, they have consolidated and formed sediment on the bottom of the aluminium sample bin showing the inability of water binding by swelling. The unchanged properties of RS2 and RS3 starches can be explained by the high amylose content. Amylose is known to have small affinity to gelatinize under cooking (Juhász and Salgó, 2008) leading to lower viscosity values in the RVA procedure. RS4 starch might be resistant against cooking due to its chemical modification leading to reversed water binding affinity and pasting properties during cooking (Xie and Liu, 2004).

The inability of resistant starches to gelatinize may lead to decreased viscosity and inconvenient textural properties of foodstuffs enriched with RS. This statement has to be taken into account in the food development and additionally the use of resistant starches must be limited and well calculated to get products with efficient properties and good consumer value.

3.2.1.2 The changes in the water absorption ability of starches

The water absorption of the starches can be greatly influenced by the heat treatment used in the food industry, hence the effects of head load was investigated and evaluated first of all in
Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta)
Timea Gelencsér, Budapest University of Technology and Economics 2009

The changes in the WA values due to the applied heat load (100 °C until 10, 20, 30 min) are summarized in Figure 22 and Table 8.

![Figure 22 Changes in the WA values of starches after heat treatment](image)

**Table 8 The changes in water absorption values due to the dry heat load**

<table>
<thead>
<tr>
<th>Starch</th>
<th>Untreated</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>83.7 ±0.5 a</td>
<td>89.0 ±0.3 b</td>
<td>93.6 ±8.7 c</td>
<td>97.5 ±8.7 c</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>81.5 ±0.5 a</td>
<td>84.4 ±0.1 b</td>
<td>87.2 ±0.3 c</td>
<td>90.2 ±0.2 d</td>
</tr>
<tr>
<td>Maize starch</td>
<td>77.6 ±2.3 a</td>
<td>82.9 ±2.3 b</td>
<td>86.4 ±1.3 c</td>
<td>91.4 ±1.8 d</td>
</tr>
<tr>
<td>Hi-maize 260</td>
<td>115.1 ±2.2 a</td>
<td>115.5 ±0.3 a</td>
<td>116.9 ±4.5 a</td>
<td>117.5 ±5.6 a</td>
</tr>
<tr>
<td>Hi-maize 1043</td>
<td>94.1 ±2.5 b</td>
<td>102.3 ±0.1 b</td>
<td>104.3 ±15.3 b</td>
<td>107.6 ±0.2 b</td>
</tr>
<tr>
<td>Novelose 330</td>
<td>190.9 ±4.1 a</td>
<td>193.0 ±0.6 a</td>
<td>196.0 ±0.1 a</td>
<td>193.8 ±24.0 a</td>
</tr>
<tr>
<td>Crystalean</td>
<td>224.9 ±3.2 a</td>
<td>226.3 ±0.3 a</td>
<td>227.4 ±0.1 a</td>
<td>227.8 ±4.8 a</td>
</tr>
<tr>
<td>C*Actistar</td>
<td>144.9 ±3.6 a</td>
<td>148.2 ±6.3 a</td>
<td>151.5 ±8.5 ab</td>
<td>151.8 ±0.2 b</td>
</tr>
<tr>
<td>Fibersym 70</td>
<td>61.1 ±5.6 a</td>
<td>70.5 ±3.5 b</td>
<td>75.2 ±1.9 c</td>
<td>76.3 ±13.4 c</td>
</tr>
</tbody>
</table>

Values represent means of triplicates±SEM
Within the same rows, the values with the same superscript are not significantly different (p <0.05)

According to the data the dry heat treatment raised small but significant changes among the native starches in the WA values in each case. The WA values of resistant starch samples were quite variable dependent on the starch type. Hi-maize 1043 and Fibersym 70 showed significant increase due to the heat load, but in the case of the other RS-s remarkable difference could not be observed after the heat treatment. This representative parameter of the starches did not seem to be strongly dependent on the applied heat load. The small changes in
the WA data could be well seen on the RVA curves as well where increased viscosity values were detected after the heat load in the case of native starches. The dry heat treatment induced small changes in the WA values for the RS samples as well; these changes however were not manifested on the RVA parameters.

3.2.1.3 Changes in the enzymatic digestibility due to dry heat load

The enzymatic digestibility of the starches is considerably dependent on the way how the starch based product was manufactured. Almost all food is heat-treated (extruded, cooked, baked etc.) before being eaten. The heating of starch containing foods can cause different changes in the crystalline structure and resistance of starches (Annison and Topping, 1994). The different methods of food processing affect the rate of starch digestion mainly under those conditions which produce obvious hydration of the granules (gelatinization). The hydration of starch during heat treatment increases its availability to amylases (Björck et al., 1994). According to our results the dry heat treatment also has significant effect on the digestibility of starches. Figure 23 and Table 9 (page 66) show the influence of heat load on the availability of starches to amylase obviously.

Figure 23 Liberated glucose before and after dry heat treatment

In most cases dry heat load caused significant increase (p<0.05) in the liberated glucose concentration. Novelose 330 and C*Actistar were the two exceptions among the starches. These RS3 starches did not seem to be dependent on the heating, their digestibility was not
sensitive to the treatment probably due to their high retrograded amylose content appearing by a previous heat load. All of the other starches showed strong dependence on the heat load time duration. Crystalean is another RS3-type starch; all the same it has denoted changes with the treatment. This starch contains mainly maltodextrin which might be sensitive to the heat load and damages have appeared probably by breakup with the rising storage temperature and time.

The duration of the storage at high temperature was a significant factor (ANOVA) and in longer period of time higher liberated glucose has appeared. The starch damages caused by the high temperature may be the most important to explain this phenomenon.

### 3.2.2 Moisture heat treatment (cooking) of native and resistant starches

As it has been earlier mentioned the digestibility of starches is significantly influenced by the applied processes in the food industry and heat treatment with hydration causes an increased availability of starches to amylases. Differences in the glycaemic response to carbohydrate meals can be brought mainly by the cooking. According to Brand et al. (1985) a much greater blood glucose response occurs after the consumption of cooked compared to raw starch. The liberated glucose concentrations of the starches are presented in Figure 24. It could be clearly observed that the cooking step caused a significant increase in the digestibility of starches in all cases. The liberated glucose of the native starches however seemed to be much higher (two or three times higher) compared to the data of the resistant starches. This phenomenon hence proved that the starches are well digested in cooked state after gelatinization.

The resistant starches showed a strong sensitivity on the moisture heat load and their availability to amylases rose significantly (p<0.05) although they did not show gelatinization in the cooking procedure similarly to the RVA measurements. The liberated glucose for the RS2 and RS4-type starches was 4-5 times as high as in the case of the untreated samples. Although for RS3-type starches this ratio was only 1.5 to 2, the lowest values were observed in the case of Hi-maize 260, Hi-maize 1043 and Fibersym 70.
Figure 24 Liberated glucose from the cooked starch samples

The total results of the heat treatment investigations are shown in Figure 25 and summarized in Table 9. The differences between the native and resistant starches are well seen as well as the differences among the resistant starches themselves.

Figure 25 Changes in the liberated glucose concentration due to the different heat treatments
Table 9 Liberated glucose concentration from starches before and after treatments

<table>
<thead>
<tr>
<th>Starches</th>
<th>Liberated glucose (mg/mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>8.10±0.33 a</td>
</tr>
<tr>
<td>Maize starch</td>
<td>5.96±0.20 a</td>
</tr>
<tr>
<td>Rice starch</td>
<td>3.18±0.50 a</td>
</tr>
<tr>
<td>Hi-maize 260</td>
<td>2.09±0.10 a</td>
</tr>
<tr>
<td>Hi-maize 1043</td>
<td>1.38±0.14 a</td>
</tr>
<tr>
<td>Novelose 330</td>
<td>4.04±0.39 a</td>
</tr>
<tr>
<td>Crystalean</td>
<td>3.62±0.30 a</td>
</tr>
<tr>
<td>C*Actistar</td>
<td>3.84±0.34 a</td>
</tr>
<tr>
<td>Fibersym 70</td>
<td>1.37±0.19 a</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM
Within the same rows, the values with the same superscript are not significantly different (p <0.05)

By evaluating the results it can be concluded that the different starches show different characteristics and properties caused by the heat load, but it has to be declared that the key step inducing high digestibility of samples is the cooking procedure.
Summarizing the results of different heat treatments on starches it can be observed that

1, the native starches are sensitive enough to show changes in the RVA due to the dry heat load while the RS-s are not influenced by the treatment and they do not gelatinize in the applied procedure. The inability of resistant starches to gelatinize may lead to decreased viscosity and inconvenient textural properties of foodstuffs enriched with RS. This statement has to be taken into account in the food development and additionally the use of resistant starches must be limited and well calculated to get products with efficient properties and good consumer value.

2, the WA values are not significantly influenced by the heat load however small changes occurred in the case of all starches leading to increased viscosity values of the native starches in the RVA procedure.

3, the enzymatic digestibility of the starches is strongly influenced by the heat load. The dry heat treatment cause significant, but definitely smaller effects than the cooking. Cooking seems to be the most determinative process influencing the availability of starches to amylases.

3.2.3 Selection of starches with adequate properties for bread and pasta

According to the results of the heat treatments and the consideration of the changes in resistance of starches; three RS-s (Hi-maize 260, Hi-maize 1043, Fibersym 70) were selected to be used in product development making bread and pasta.

In the next chapters the results of the investigations of the bread and pasta samples are summarized and evaluated.
3.3 Bread making with resistant starch addition and the comparison of the products

3.3.1 Rheological properties of bread raw materials measured by RVA method

The rheological properties of bread raw materials were significantly influenced by the RS addition shown in Figure 26. There was a decrease in the viscosity values of samples after the RS inclusion in all cases. The results highlight the negative influence of resistant starches on the physico-chemical properties of bread raw materials.

![Figure 26 RVA curves of bread raw materials](image)

The typical parameters of the curves summarized in Table 10 also represent the changed gelatinization characteristic of samples caused by the RS components.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PV(cP)</th>
<th>TR(cP)</th>
<th>BD(cP)</th>
<th>FV(cP)</th>
<th>SB(cP)</th>
<th>PT(min)</th>
<th>PTp(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL55 flour</td>
<td>1612.0</td>
<td>658.5</td>
<td>953.5</td>
<td>1471.5</td>
<td>813.0</td>
<td>5.6</td>
<td>67.0</td>
</tr>
<tr>
<td>BL55+Hi-maize260</td>
<td>801.0</td>
<td>318.5</td>
<td>482.5</td>
<td>718.5</td>
<td>400.0</td>
<td>5.2</td>
<td>84.3</td>
</tr>
<tr>
<td>BL55+Hi-maize1043</td>
<td>887.0</td>
<td>345.0</td>
<td>542.0</td>
<td>762.5</td>
<td>417.5</td>
<td>5.2</td>
<td>83.9</td>
</tr>
<tr>
<td>BL55+Fibersym70</td>
<td>899.0</td>
<td>364.0</td>
<td>535.0</td>
<td>885.5</td>
<td>521.5</td>
<td>5.2</td>
<td>84.4</td>
</tr>
</tbody>
</table>

PV peak viscosity, TR trough, BD breakdown, FV final viscosity, SB setback, PT peak time, PTp pasting temperature
Values represent means of duplicates
The viscosity parameters (peak viscosity, trough, breakdown, final viscosity and setback) were significantly (p<0.05) lowered by the resistant starches, moreover the other two parameters (peak time and pasting temperature) also showed statistically confirmed alterations (p<0.05). The viscosity of RS-added samples was reduced by 40-50% applied the starches in 20%. This very notable effect indicates the strong influence of RS-s on the physical and sensorial properties of baked products as well.

### 3.3.2 Physical and sensory properties of bread products

After the baking under standard conditions, bread products were investigated from the weight and volume values point of view as well as sensory properties and digestibility characters. The physical properties are shown in Table 11 where it can be well observed that the resistant starch addition did not cause significant differences in the weight of products. The volume of the RS-added samples however was significantly (p<0.05) lower compared to the control bread. The RS and gluten addition may induce an inefficient texture of the crumb due to an inadequate integration into the bread structure.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight (g)</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control bread</td>
<td>352±4⁸</td>
<td>1025±7⁹</td>
</tr>
<tr>
<td>Bread with A starch</td>
<td>355±0⁹</td>
<td>940±14⁹</td>
</tr>
<tr>
<td>Bread with B starch</td>
<td>350±0⁹</td>
<td>935±35⁹</td>
</tr>
<tr>
<td>Bread with C starch</td>
<td>350±14⁹</td>
<td>895±7⁹</td>
</tr>
</tbody>
</table>

Values represent means of triplicates±SEM
Within the same column, the values with the same superscript are not significantly different (p <0.05)

The differences among the products can be followed in Figure 27a and b. The height of the products has decreased by the RS addition indicating the lower volume for these products. The crumb of the products has been also influenced by the RS inclusion. There were some starch clusters in each RS enriched product showing the inefficient integration of resistant starches into the bread structure. The crumb of these samples was clammy and wet after the baking.

The sensory values of the crumb however were not significantly different compared to the control product based on the evaluation of the test panel (Table 12).
Figure 27a Baked products

Figure 27b The crumb of bread samples
According to the sensory panel each bread product could be ranged into the first category (excellent). Although the bread enriched with Hi-maize 1043 (C starch) seemed to be less preferred by the sensory panel. Accordingly it had significantly (p<0.05) lower total score compared to the other products presented in Table 12.

Table 12 Results of sensory evaluation of bread products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shape</th>
<th>Crust</th>
<th>Crumb</th>
<th>Smell</th>
<th>Taste</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control bread</td>
<td>5.0±0.0ᵃ</td>
<td>4.7±0.2ᵃ</td>
<td>4.2±0.2ᵇ</td>
<td>4.7±0.2ᵃ</td>
<td>4.8±0.1ᵃ</td>
<td>18.4±0.5ᵇ</td>
</tr>
<tr>
<td>Bread with A starch</td>
<td>5.0±0.0ᵃ</td>
<td>4.3±0.2ᵇ</td>
<td>4.4±0.2ᵃ</td>
<td>4.3±0.2ᵇ</td>
<td>4.3±0.2ᵇ</td>
<td>17.9±0.7ᵇ</td>
</tr>
<tr>
<td>Bread with B starch</td>
<td>5.0±0.0ᵃ</td>
<td>4.7±0.1ᵃ</td>
<td>4.6±0.2ᵃ</td>
<td>4.7±0.2ᵃ</td>
<td>4.7±0.2ᵃ</td>
<td>18.7±0.5ᵃ</td>
</tr>
<tr>
<td>Bread with C starch</td>
<td>5.0±0.0ᵇ</td>
<td>3.9±0.8ᵇ</td>
<td>3.8±0.7ᵇ</td>
<td>4.0±0.9ᵇ</td>
<td>4.1±0.8ᵇ</td>
<td>16.3±2.4ᵇ</td>
</tr>
</tbody>
</table>

Values represent means of 10 points ±SEM
Within the same columns, the values with the same superscript are not significantly different (p <0.05)

It can be concluded that bread with RS addition may have similar properties than the commercial (control) product; resistant starches do not change the organoleptic properties of samples, the volume and the crumb texture however are affected in an unfavourable way; although the extent of this effect is acceptable from the sensory properties point of view.

3.3.3 Enzymatic digestion and resistant starch content of bread products

The digestion curves of the tested materials were analysed as the solution of first-order kinetics, according to Goñi et al. (1997). The nonlinear equation used for the evaluations was the following

\[ C = C_{\text{max}} (1 - e^{-kt}) \]

where \( C \) is the concentration at \( t \) time, \( C_{\text{max}} \) is the equilibrium concentration, \( k \) is the rate constant and \( t \) is the sampling time. A comprehensive parameter for the digestibility was also calculated as the total area under curve (AUC, mg glucose/g sample × min) relating to glucose release over the whole test period (180 min).

Due to the great influence of baking on the starch digestibility (Annison and Topping, 1994), the aim of the measurements was to investigate the effects of resistant starches on the liberated glucose from the raw dough and the baked bread products as well as to study the decrease in the RS content of samples after baking.
The kinetics of raw dough samples are presented in Figure 28.

![Figure 28 Kinetic curves of raw bread dough samples](image)

The fitted first order kinetic curves differed only in a small compass according to the graph. The resistant starch addition did not cause the change of the running and the characteristic of the kinetic curves. This statement was confirmed by the kinetic parameters as well which are collected in Table 13.

**Table 13 Kinetic parameters of bread dough samples before baking**

<table>
<thead>
<tr>
<th>Sample</th>
<th>C_{max} (mg/g)</th>
<th>k (1/min)</th>
<th>AUC (mg/g) x min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control raw bread</td>
<td>5.94±0.16^a</td>
<td>0.045±0.003^a</td>
<td>920.50±17.4^a</td>
</tr>
<tr>
<td>Bread dough with A starch</td>
<td>5.87±0.15^a</td>
<td>0.035±0.002^b</td>
<td>875.53±11.25^b</td>
</tr>
<tr>
<td>Bread dough with B starch</td>
<td>5.81±0.05^b</td>
<td>0.041±0.003^ab</td>
<td>890.93±0.55^b</td>
</tr>
<tr>
<td>Bread dough with C starch</td>
<td>5.80±0.09^b</td>
<td>0.040±0.003^ab</td>
<td>887.20±4.78^b</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM
Within the same column, the values with the same superscript are not significantly different (p <0.05)

The values of C_{max} and k did not significantly differ among the RS-added and control dough samples. The AUC rates were contrarily different for the RS-enriched and control raw products. The resistant starches caused remarkable decrease in the AUC. The appearing reducing effect was rather small (4-5 % set against the control dough) but it was statistically supported. According to these results, it can be noticed that resistant starches had slight influence on the digestibility of bread dough.

As it has emerged previously, baking procedure has remarkable effect on the properties of bread products as well as the amount of resistant starches. The high-amylose starches (A and C) may be sensitive on heat treatment while there is not enough information about the changes of chemically modified starches during baking. After the heat treatment, however, novel resistant starch component namely retrograded amylose can emerge and increase the
resistant starch content of products (Tas and El, 2000). The kinetic curves of bread samples are introduced in Figure 29 and the parameters summarized in Table 14.

![Figure 29 Kinetic curves of bread samples](image)

**Table 14 Kinetic parameters of bread products**

<table>
<thead>
<tr>
<th>Sample</th>
<th>( C_{\text{max}} ) (mg/g)</th>
<th>( k ) (1/min)</th>
<th>AUC (mg/g) * min</th>
<th>Relative digestibility (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control bread</td>
<td>7.94±0.09b</td>
<td>0.139±0.052c</td>
<td>1304.33±25.79c</td>
<td>100</td>
</tr>
<tr>
<td>Bread with A starch</td>
<td>7.42±0.21c</td>
<td>0.064±0.100d</td>
<td>1182.00±17.35d</td>
<td>90.62</td>
</tr>
<tr>
<td>Bread with B starch</td>
<td>8.01±0.27b</td>
<td>0.077±0.020d</td>
<td>1266.33±6.11c</td>
<td>97.09</td>
</tr>
<tr>
<td>Bread with C starch</td>
<td>7.87±0.13b</td>
<td>0.072±0.010d</td>
<td>1270.33±4.81c</td>
<td>97.39</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM  
Within the same column, the values with the same superscript are not significantly different (p <0.05)  
†represents ratio of AUC_REnriched bread/AUC контроль bread

Investigating the results it was observed that the values of \( C_{\text{max}} \) and \( k \) were not significantly different for the RS-added products compared to the conventional (control) sample. At the same time, the AUC was reduced remarkably in the case of Hi-maize 260 (A starch) added bread proved by the Figure 30. Results have also confirmed the effects of baking on the digestibility (Figure 30). The \( C_{\text{max}} \) and AUC parameters were significantly higher (p<0.05) for baked products compared to the raw dough samples. The increase of the absolute glucose release characterised by the AUC had an extent of about 35-40% (Table 13 and 14). The relative digestibility, referring to the decrease in digestibility, can be calculated as the ratio of AUC_REnriched bread /AUC контроль bread. The relative digestibility seemed to be smaller for all RS-added bread samples, the highest decrease in the digestibility could be observed in the case of A starch (Hi-maize 260). The decrease in the digestibility of this bread could prove the appearance of retrograded starch (Tas and El, 2000) during the short storage before analysis.
Figure 30 Differences between the dough and the bread samples

The $k$ values were significantly higher for the bread samples compared to the proper dough indicating a faster glucose release after baking. It can prove the results of previous studies that bread products are digested rapidly after consumption leading to high glucose level in the blood after a short period of time (Åkerberg et al., 1998). The relative digestibility values for RS added products were lower compared to the control bread; the extent of this decrease however was only notable in the case of Hi-maize 260 included sample.

There were big changes in the RS content of all products as well. According to Åkerberg et al. (1998) bread products usually contain 1-5 % resistant starch measured in vitro dependent on the flour and baking conditions. Our results showed good correlation with this statement as a resistant starch content of 4.98-6.43 % was measured in the products (Table 15).
Table 15 Resistant starch content of raw and baked samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>RS content (w/w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control dough</td>
<td>16.74±0.5c</td>
</tr>
<tr>
<td>Bread dough with A starch</td>
<td>17.98±0.65c</td>
</tr>
<tr>
<td>Bread dough with B starch</td>
<td>17.49±0.67c</td>
</tr>
<tr>
<td>Bread dough with C starch</td>
<td>17.05±0.71c</td>
</tr>
<tr>
<td>Control bread</td>
<td>4.98±0.17a</td>
</tr>
<tr>
<td>Bread with A starch</td>
<td>6.18±0.40b</td>
</tr>
<tr>
<td>Bread with B starch</td>
<td>6.43±0.15b</td>
</tr>
<tr>
<td>Bread with C starch</td>
<td>5.53±0.26b</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM
Within the same column, the values with the same superscript are not significantly different (p <0.05)

The baking step had significant (p<0.05) effect on the RS content of samples. The resistant starch content of raw dough samples was about 17 % which has decreased significantly after baking. Accordingly it can be stated that heat treatment cause remarkable changes in the RS content of starch-based products. The resistant starches used in the measurements seemed to be heat sensitive, although the RS content of resistant starch included products was higher compared to the control bread. The difference between the Hi-maize 1043 (C starch) added and control product was not significant.

The results of these studies indicate the fact that due to the strong heat sensitivity of resistant starches they can be digested by amylolytic enzymes after baking more rapidly, and only a small amount of starch remains resistant. In higher replacement of flour there could be a more notable RS content after baking, at the same time the higher RS content should lead to weak bread structure and inefficient sensory properties.

Summarizing the results of bread baking using RS additives in the dough it can be stated that

1, the use of resistant starches can negatively influence the physical properties of bread samples mainly the volume of the products. The RS addition cause inhomogeneous, wet crumb structure containing starch clusters deteriorating the consumer value of the products.

2, the sensory properties of the samples were not significantly different according to the control panel, the taste of the RS added products however was less preferred than that of the control sample.
3, the RS-s did not affect the digestibility of dough and bread samples significantly (except Hi-maize 260), moreover the RS content of the samples was radically reduced by the baking.

4, the changes in the RS content of samples highlight the difficulties of the use RS addition in bread samples. The resistant starches show namely strong heat sensitivity and loose their resistance during the baking process. The baking at the same time can lead to the appearance of retrograded starch in the samples indicating higher RS content after a short storage.

Following the experiments with bread samples, in the next research phase pasta samples were produced with RS addition. The aim of the studies was to evaluate the effects of resistant starches on the rheological properties of pasta samples as well as the digestibility of raw mixtures, extruded samples and cooked products. The changes in the RS content of pasta due to the applied process were also investigated.
3.4 Pasta production with resistant starch addition and the comparison of the products

3.4.1 Rheological properties of pasta raw materials using the RVA method

The rheological properties of pasta products were notably influenced by the RS addition (Figure 31). According to the properties of resistant starches, a decreasing effect in the viscosity parameters could be expected. The results also indicated this possibility and RS-s had significant effects on the physico-chemical properties of pasta products. The typical viscosity parameters decreased substantially with the addition of RS compared to the control pasta sample respectively. Figure 31 shows that the character and the time-course of the viscosity curves are similar in all pasta products indicating the negligible effect of resistant starches on the running of the curves. The RS-s caused similar decreases in the viscosity parameters therefore only the pastas with the A starch (Hi-maize 260) are shown here.

Table 16 shows the typical RVA parameters for the pasta raw materials. The rheology of pasta products was significantly influenced by the type of flour used in the sample (ANOVA). The lower absolute values of viscosities have been caused clearly by the composition of dough.
Table 16 Changes in the RVA parameters

<table>
<thead>
<tr>
<th>Pasta</th>
<th>PV(cP)</th>
<th>TR(cP)</th>
<th>BD(cP)</th>
<th>FV(cP)</th>
<th>SB(cP)</th>
<th>PT(min)</th>
<th>PTp(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. aestivum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control pasta</td>
<td>2181.0</td>
<td>1059.0</td>
<td>1122.0</td>
<td>2412.0</td>
<td>1353.0</td>
<td>5.4</td>
<td>81.5</td>
</tr>
<tr>
<td>AA10</td>
<td>1685.0</td>
<td>786.5</td>
<td>898.5</td>
<td>1781.0</td>
<td>994.5</td>
<td>5.3</td>
<td>82.3</td>
</tr>
<tr>
<td>AA20</td>
<td>1212.0</td>
<td>601.0</td>
<td>611.0</td>
<td>1360.0</td>
<td>759.0</td>
<td>5.2</td>
<td>82.8</td>
</tr>
<tr>
<td>AB10</td>
<td>1705.0</td>
<td>822.0</td>
<td>883.0</td>
<td>1852.0</td>
<td>1030.0</td>
<td>5.4</td>
<td>82.0</td>
</tr>
<tr>
<td>AB20</td>
<td>1256.0</td>
<td>652.2</td>
<td>603.5</td>
<td>1513.0</td>
<td>860.5</td>
<td>5.2</td>
<td>83.6</td>
</tr>
<tr>
<td>AC10</td>
<td>1712.5</td>
<td>859.5</td>
<td>853.0</td>
<td>1982.0</td>
<td>1122.5</td>
<td>5.2</td>
<td>82.7</td>
</tr>
<tr>
<td>AC20</td>
<td>1313.5</td>
<td>646.0</td>
<td>667.5</td>
<td>1469.5</td>
<td>823.5</td>
<td>5.2</td>
<td>82.8</td>
</tr>
<tr>
<td>T. durum</td>
<td>1297.5</td>
<td>596.5</td>
<td>701.0</td>
<td>1399.0</td>
<td>802.5</td>
<td>5.2</td>
<td>81.5</td>
</tr>
<tr>
<td>control pasta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA10</td>
<td>857.5</td>
<td>397.0</td>
<td>460.5</td>
<td>954.0</td>
<td>557.0</td>
<td>5.1</td>
<td>85.2</td>
</tr>
<tr>
<td>DA20</td>
<td>541.0</td>
<td>275.5</td>
<td>265.5</td>
<td>636.0</td>
<td>360.5</td>
<td>5.0</td>
<td>85.9</td>
</tr>
<tr>
<td>DB10</td>
<td>848.5</td>
<td>371.5</td>
<td>477.0</td>
<td>949.0</td>
<td>577.5</td>
<td>5.1</td>
<td>82.4</td>
</tr>
<tr>
<td>DB20</td>
<td>607.5</td>
<td>286.5</td>
<td>321.0</td>
<td>731.0</td>
<td>444.5</td>
<td>5.1</td>
<td>77.9</td>
</tr>
<tr>
<td>DC10</td>
<td>925.5</td>
<td>416.5</td>
<td>509.0</td>
<td>1021.0</td>
<td>604.5</td>
<td>5.2</td>
<td>83.3</td>
</tr>
<tr>
<td>DC20</td>
<td>631.0</td>
<td>321.0</td>
<td>310.0</td>
<td>770.0</td>
<td>449.0</td>
<td>5.1</td>
<td>86.8</td>
</tr>
</tbody>
</table>

PV peak viscosity, TR trough, BD breakdown, FV final viscosity, SB setback, PT peak time, PTp pasting temperature
Values represent means of duplicates

The effect of added RS-s was similar in both *T. aestivum* and *T. durum* products causing linear, but not stoichiometric reduction in the typical RVA parameters. As RS-s were not able to gelatinize in the procedure, these decreased values have been expected. Not only the type of RS but also the added amount showed significant effect (ANOVA, p<0.05) on the representative parameters. Among the seven typical RVA parameters there were five which could express the changes in the viscoamylographic characteristics of the pastas. These were the peak viscosity, trough, breakdown, final viscosity and setback. The parameters of *T. aestivum* samples showed a decrease of 17 % to 26.5 % in the case of 10 % resistant starch addition and 37.3 % to 46.2 % in the case of 20 % resistant starch addition into the pasta mixtures. The decrease was slightly higher in the case of *T. durum* pasta products. The addition of 10 % starch caused about 24.6 to 37.7 % reduction in the parameters while a decrease of 44 to 62.1 % could be observed in the presence of 20 % resistant starch. The different changes between the two groups of samples (*T. aestivum, T. durum*) may occur due to the different flour and interactions among the flour components and resistant starches used in the processing. Peak time and pasting temperature did not seem to be sensitive enough to follow up the viscoamylographic changes. According to the RVA results changes in the cooking properties caused by the RS addition and the difference between the two basic materials (*T. aestivum* or *T. durum*) were expected.
3.4.2 Comparison of cooking properties of different pasta products

The cooking properties of the pasta samples are shown in Table 17.

Table 17 Cooking properties of different pasta products

<table>
<thead>
<tr>
<th>Pasta</th>
<th>Water absorption (%)</th>
<th>Cooking loss (%)</th>
<th>Swelling index</th>
<th>Dry matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>151.5±2.3&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>3.9±0.1&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>1.9±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA10</td>
<td>157.0±1.7&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>3.5±0.1&lt;sup&gt;bcef&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.0±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA20</td>
<td>160.0±1.6&lt;sup&gt;c e&lt;/sup&gt;</td>
<td>4.1±0.3&lt;sup&gt;adde&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.6±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AB10</td>
<td>154.6±3.0&lt;sub&gt;ac&lt;/sub&gt;</td>
<td>3.7±0.1&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>1.9±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.2±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AB20</td>
<td>149.0±1.8&lt;sup&gt;ade&lt;/sup&gt;</td>
<td>4.2±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.7±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC10</td>
<td>153.1±0.9&lt;sub&gt;af&lt;/sub&gt;</td>
<td>3.4±0.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.9±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.4±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC20</td>
<td>154.1±2.1&lt;sup&gt;af&lt;/sup&gt;</td>
<td>3.7±0.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.9±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.0±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D control</td>
<td>149.4±3.9&lt;sub&gt;abgh&lt;/sub&gt;</td>
<td>5.2±0.3&lt;sup&gt;a&lt;/sub&gt;</td>
<td>1.9±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.4±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DA10</td>
<td>143.5±4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8±0.2&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.9±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.0±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DA20</td>
<td>148.7±2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7±0.2&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.9±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.5±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB10</td>
<td>148.7±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±0.2&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.0±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB20</td>
<td>144.2±1.8&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>5.8±0.1&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.8±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC10</td>
<td>152.8±1.1&lt;sup&gt;beth&lt;/sup&gt;</td>
<td>5.8±0.3&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC20</td>
<td>158.1±3.2&lt;sup&gt;bh&lt;/sup&gt;</td>
<td>6.1±0.1&lt;sup&gt;bv&lt;/sup&gt;</td>
<td>1.8±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.7±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent means of 4 replicates ± SEM. Within the same column, the values with the same superscript are not significantly different (p<0.05). * represents values significantly different from the control pasta (Dunnett’s test)

Results indicated that there were no significant differences in the swelling index and dry matter data between RS added and control products (according to Dunnett’s tests). Swelling index values ranged from 1.8 to 1.9 for both T. aestivum and T. durum pasta products. The type of flour, the type of RS and the amount of added RS-s (10% or 20%) did not seem to have any effect on these two cooking parameters.

Cooking loss is commonly used to predict pasta cooking performance. Table 17 illustrates that cooking losses differed significantly (p<0.05) between the two groups of pasta (T. durum and T. aestivum) samples (accordingly the type of flour had significant effect on this measured parameter proved by ANOVA). The values ranged from 3.4 % to 4.2 % for the T. aestivum and from 5.2 % to 6.1 % for the T. durum pastas. The apparent lower cooking loss in the case of T. aestivum pastas may have occurred due to the egg powder additive which could help to maintain and strengthen the pasta structure during cooking. Egg powder can act as an adhesive in the pasta. The higher cooking losses of the T. durum pastas could be explained by the easier disruption of the protein network during the cooking process. The protein matrix
usually disintegrates continuously, releasing exudates during starch granule gelatinization, which results an increase in cohesiveness and stickiness on the surface of the cooked pasta (Tudorică et al., 2002). This phenomenon might have been delayed by egg powder addition in *T. aestivum* products. Among the *T. aestivum* samples only Hi-maize 1043 of 10 % showed a significant effect, the other pasta samples were not significantly different from the control pasta. Comparing the *T. durum* samples it could be seen that all RS-s had significant increasing effect on the cooking loss value (p<0.05).

The type of flour had significant effect (p<0.05) also on the water absorption of pasta samples. Evaluating the water absorption data it could be seen that among *T. aestivum* products, only Hi-maize 260 at 20 % addition resulted in a significant increase. Among the *T. durum* products, the RS addition did not cause significant effect compared to the proper control pasta (according to Dunnett’s test). To sum up the results of cooking tests, it can be concluded that RS-s did not affect the characteristics of pasta products during the cooking procedure notably in spite of the expectations after the RVA measurements.

### 3.4.3 Sensory properties of different pasta products

The results of sensory tests are summarised in Table 18. According to the statistics pastas enriched with RS-s were not significantly different from the proper *T. aestivum* or *T. durum* control samples. The type of flour used in the process seemed to be however very important. The weighted total score for the *T. durum* products was significantly lower (p<0.05) compared to the pastas produced from *T. aestivum* wheat flour. It should be noted, however, that all of the products could be ranged into the first quality category (total score is above 16). The appearance and texture of the products did not have big variance, the flour, RS type and amount of starch did not seem to cause changes in these parameters according to the sensory panel. The smell and taste were less preferred for the *T. durum* products, therefore it can be stated that the flour type caused significant difference among the samples. Evaluating the preference test it can be declared that pastas with RS-s did not differ significantly from the common commercial products.
Table 18 Results of sensory evaluation of samples

<table>
<thead>
<tr>
<th>Pasta</th>
<th>Appearance</th>
<th>Smell</th>
<th>Taste</th>
<th>Texture</th>
<th>Weighed total score</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. aestivum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control pasta</td>
<td>4.6±0.2a</td>
<td>4.9±0.1a</td>
<td>4.8±0.1a</td>
<td>4.7±0.2a</td>
<td>18.9±0.5a</td>
</tr>
<tr>
<td>AA10</td>
<td>4.5±0.2a</td>
<td>4.7±0.2a</td>
<td>4.7±0.2a</td>
<td>4.5±0.2a</td>
<td>18.3±0.5a</td>
</tr>
<tr>
<td>AA20</td>
<td>4.4±0.2a</td>
<td>4.9±0.1a</td>
<td>4.6±0.2a</td>
<td>4.5±0.2a</td>
<td>18.3±0.4a</td>
</tr>
<tr>
<td>AB10</td>
<td>4.5±0.2a</td>
<td>4.6±0.2a</td>
<td>4.5±0.2a</td>
<td>4.5±0.3a</td>
<td>18.1±0.7a</td>
</tr>
<tr>
<td>AB20</td>
<td>4.5±0.2a</td>
<td>4.9±0.1a</td>
<td>4.4±0.2a</td>
<td>4.9±0.1a</td>
<td>18.7±0.3a</td>
</tr>
<tr>
<td>AC10</td>
<td>4.8±0.1a</td>
<td>4.9±0.1a</td>
<td>5.0±0.1a</td>
<td>4.4±0.2a</td>
<td>18.9±0.2a</td>
</tr>
<tr>
<td>AC20</td>
<td>4.7±0.2a</td>
<td>5.0±0.0a</td>
<td>5.0±0.0a</td>
<td>4.3±0.3a</td>
<td>18.8±0.5a</td>
</tr>
<tr>
<td><em>T. durum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control pasta</td>
<td>4.5±0.2a</td>
<td>4.7±0.2a</td>
<td>4.5±0.2a</td>
<td>4.7±0.2a</td>
<td>18.4±0.4a</td>
</tr>
<tr>
<td>DA10</td>
<td>4.8±0.1a</td>
<td>4.3±0.2b</td>
<td>3.8±0.2b</td>
<td>4.8±0.1a</td>
<td>17.7±0.4b</td>
</tr>
<tr>
<td>DA20</td>
<td>4.6±0.2a</td>
<td>4.3±0.3b</td>
<td>3.9±0.2b</td>
<td>4.7±0.2a</td>
<td>17.5±0.6b</td>
</tr>
<tr>
<td>DB10</td>
<td>4.6±0.2a</td>
<td>4.1±0.2b</td>
<td>4.3±0.3b</td>
<td>4.9±0.1b</td>
<td>17.9±0.5b</td>
</tr>
<tr>
<td>DB20</td>
<td>4.3±0.2a</td>
<td>4.4±0.2b</td>
<td>4.2±0.3b</td>
<td>4.3±0.2a</td>
<td>17.2±0.7b</td>
</tr>
<tr>
<td>DC10</td>
<td>4.4±0.2a</td>
<td>4.1±0.3b</td>
<td>4.2±0.2b</td>
<td>4.7±0.2a</td>
<td>17.4±0.5b</td>
</tr>
<tr>
<td>DC20</td>
<td>4.6±0.2a</td>
<td>4.3±0.3b</td>
<td>4.2±0.2b</td>
<td>4.7±0.2a</td>
<td>18.1±0.5a</td>
</tr>
</tbody>
</table>

Values represent means of 10 scores ±SEM.
Within the same column, the values with the same superscript are not significantly different (p<0.05)

3.4.4 Enzymatic digestion and resistant starch content of pasta products

The digestion curves of the tested materials were analysed as the solution of first-order kinetics, according to Goñi et al. (1997). The nonlinear equation used for the evaluations was the following

$$C = C_{max} (1-e^{-kt})$$

where $C$ is the concentration at $t$ time, $C_{max}$ is the equilibrium concentration, $k$ is the rate constant and $t$ is the sampling time. A comprehensive parameter for the digestibility was also calculated as the total area under curve ($AUC$, (mg glucose/g sample)× min) relating to glucose release over the whole test period (180 min).

3.4.4.1 Enzymatic digestion of raw pasta mixtures

Figure 32 shows the kinetics of raw *T. aestivum* mixtures before treatments (extrusion and cooking). The graph confirmed that the resistant starches did not cause any changes in the running and the shape of the curves; all samples followed the first order kinetics perfectly ($R^2>0.98$).
The typical kinetic parameters ($C_{\text{max}}$, $k$, AUC) of the samples are presented in Table 19.

### Table 19 Kinetic parameters of *T. aestivum* mixtures

<table>
<thead>
<tr>
<th>Sample</th>
<th>$C_{\text{max}}$ (mg/g)</th>
<th>$k$ (1/min)</th>
<th>AUC (t=180 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>5.35±0.18 $^a$</td>
<td>0.026±0.003 $^a$</td>
<td>752.17±13.76 $^a$</td>
</tr>
<tr>
<td>AA10</td>
<td>4.85±0.21 $^a$</td>
<td>0.024±0.003 $^a$</td>
<td>664.20±7.05 $^b$</td>
</tr>
<tr>
<td>AB10</td>
<td>5.35±0.25 $^a$</td>
<td>0.020±0.003 $^a$</td>
<td>703.03±7.65 $^c$</td>
</tr>
<tr>
<td>AC10</td>
<td>5.70±0.32 $^a$</td>
<td>0.018±0.003 $^a$</td>
<td>719.97±4.86 $^d$</td>
</tr>
<tr>
<td>AA20</td>
<td>5.11±0.25 $^a$</td>
<td>0.022±0.003 $^a$</td>
<td>683.53±3.09 $^e$</td>
</tr>
<tr>
<td>AB20</td>
<td>5.35±0.25 $^a$</td>
<td>0.019±0.002 $^a$</td>
<td>681.87±13.86 $^e$</td>
</tr>
<tr>
<td>AC20</td>
<td>5.43±0.26 $^a$</td>
<td>0.020±0.003 $^a$</td>
<td>704.37±4.29 $^e$</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same column, the values with the same superscript are not significantly different (p<0.05)

For the *T. aestivum* mixtures $C_{\text{max}}$ values did not differ significantly according to the T-tests, moreover the mixtures with RS did not vary in compared to the control mixture confirmed by the Dunnett’s tests. The values are within a narrow interval (4.85 – 5.70 mg glucose/g sample), the resistant starch addition accordingly did not have any notable influence on the equilibrium concentration parameter. The kinetic constant ($k$) contrarily showed a more remarkable variance. For the *T. aestivum* samples a small decrease could be noticed due to the resistant starch addition, this effect, however, was not significant. The resistant starch-added mixtures whereas reached smaller AUC values in each case compared to the control sample. The differences were statistically confirmed, thus it follows from this that the resistant starches caused a significant (p<0.05) reduction in the absolute glucose release (digestibility) but did not have any effects on the shape and characteristics of kinetics in the case of raw *T. aestivum* mixtures.
Figure 33 shows the kinetics of raw *T. durum* mixtures before the treatments. In connection with the graph and the kinetic parameters summarized in Table 20 similar conclusions can be stated as in the case of the *T. aestivum* samples.

![Figure 33 Kinetic curves of raw T. durum mixtures](image)

Table 20 Kinetic parameters of different raw mixtures

<table>
<thead>
<tr>
<th>Sample</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/g)</th>
<th>k (1/min)</th>
<th>AUC (t=180 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D control</td>
<td>5.53±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.032±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>816.27±16.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DA10</td>
<td>5.82±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>808.20±20.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB10</td>
<td>5.71±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.022±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>760.67±8.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC10</td>
<td>5.79±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.021±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>770.37±9.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DA20</td>
<td>5.42±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.027±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>769.70±6.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB20</td>
<td>5.41±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.023±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>730.20±8.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC20</td>
<td>5.56±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.017±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>686.03±20.13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same column, the values with the same superscript are not significantly different (p<0.05). * represents values significantly different from the control pasta (Dunnett’s test).

The resistant starches did not cause any changes in the running and the shape of the curves moreover all samples followed the first order kinetics perfectly (R<sup>2</sup> > 0.98). The equilibrium concentration was not influenced by the RS addition, the kinetic constant, was decreased in most cases (DB10, DC10, DB20 and DC20), and the AUC was significantly (p<0.05) lowered by the RS addition (except from DA10). Accordingly it could be stated that the A starch had the least effect on the digestion parameters. The analysis of the kinetic parameters of the *T. durum* raw samples shows that the resistant starch addition may slightly influence the digestibility of mixtures but does not have any effects on the kinetics of the amylolysis.
3.4.4.2 Enzymatic digestion of extruded and dried pasta samples

Figure 34 illustrates the kinetics of the extruded and dried *T. aestivum* samples. The fitted lines of the samples represent that the difference between the RS-added and control pasta samples is quite large. The shape of the curves, however, was not affected by the RS addition and the fitted lines followed the first order kinetics properly (R²>0.98).

![Figure 34 Kinetic curves of extruded *T. aestivum* samples](image)

According to Table 21 the extrusion had significant effect (p<0.05) on the equilibrium concentration and on the AUC values based on ANOVA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/g)</th>
<th>k (1/min)</th>
<th>AUC (t=180 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>6.74±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>932.73±54.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA10</td>
<td>5.93±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.023±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>805.23±11.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AB10</td>
<td>6.15±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.023±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>829.37±22.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC10</td>
<td>6.43±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.022±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>857.17±2.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA20</td>
<td>5.79±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.019±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>739.93±17.79&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>AB20</td>
<td>5.60±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.023±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>756.97±26.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC20</td>
<td>5.93±0.15&lt;sup&gt;b&lt;/sup&gt;c</td>
<td>0.025±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>823.57±2.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same column, the values with the same superscript are not significantly different (p<0.05)

The C<sub>max</sub> and AUC values were increased markedly for all *T. aestivum* samples after the process compared to the proper raw mixtures (Table 19 and 21). The highest digestibility (AUC = 932.7) was possessed by the control sample. This observation confirmed the extended availability of starches to α-amylase after the conventional, standard extrusion step. The kinetic constant parameter (k) was not significantly different among the RS-added and
control samples. Moreover the velocity of digestibility was not influenced by the extrusion process.

There were big differences among the fitted kinetic curves of *T. durum* extruded samples demonstrated by Figure 35. The shape of the curves was not significantly affected by the RS addition, the $R^2$ values, however, were smaller in the case of RS added ($R^2 =0.96$) compared to the control sample ($R^2=0.98$).

![Figure 35](image)

**Figure 35** Kinetic curves of extruded *T. durum* samples

The extrusion step did not have significant effects on the $C_{\text{max}}$ values reported in Table 22, compared to the raw mixtures (Table 20). The *T. durum* samples seemed to be more resistant against the applied extrusion conditions than the *T. aestivum* samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$C_{\text{max}}$ (mg/g)</th>
<th>$k$ (1/min)</th>
<th>AUC (t=180 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D control</td>
<td>5.80±0.18 $^a$</td>
<td>0.025±0.003 $^a$</td>
<td>800.50±3.60 $^a$</td>
</tr>
<tr>
<td>DA10</td>
<td>5.15±0.25 $^a$</td>
<td>0.024±0.004 $^a$</td>
<td>703.23±17.99 $^b$</td>
</tr>
<tr>
<td>DB10</td>
<td>5.64±0.21 $^a$</td>
<td>0.023±0.003 $^a$</td>
<td>766.03±20.12 $^c$</td>
</tr>
<tr>
<td>DC10</td>
<td>5.59±0.20 $^a$</td>
<td>0.024±0.003 $^a$</td>
<td>769.10±11.36 $^c$</td>
</tr>
<tr>
<td>DA20</td>
<td>5.58±0.14 $^a$</td>
<td>0.037±0.003 $^b$</td>
<td>842.30±30.05 $^d$</td>
</tr>
<tr>
<td>DB20</td>
<td>5.85±0.18 $^a$</td>
<td>0.030±0.004 $^{ab}$</td>
<td>844.27±4.09 $^d$</td>
</tr>
<tr>
<td>DC20</td>
<td>5.46±0.21 $^a$</td>
<td>0.021±0.002 $^a$</td>
<td>722.00±9.85 $^b$</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM.
Within the same column, the values with the same superscript are not significantly different (p<0.05)

The $k$ value was not significantly different among the samples (DA20 was exceptional). Thus it can be concluded that the kinetic constant was not influenced by the extrusion, similarly to the *T. aestivum* samples. The AUC-s were quite variable among the *T. durum* samples after
the extrusion in one case it caused a small decrease (DA10) in some cases did not cause any changes (D control, DB10, DC10) and in the rest cases (DA20, DB20, DC20) an increase could be observed after the process.

By evaluating the changes after extrusion our conclusion was that this technological process caused alterations in the digestibility of all products this alteration however was not significant in all cases. The extent and type of changes were dependent on the flour used in the pasta as well as the properties of resistant starches added into the products.

3.4.4.3 Enzymatic digestion of cooked pasta samples

The kinetic curves of RS-enriched and control pasta products after cooking are shown in Figure 36. The typical kinetic parameters are summarized in Table 23. In Figure 36 it can be clearly seen that the RS addition did not cause significant changes in the kinetics. The $R^2$ values of the fitted curves were above 0.98 in all cases which could prove that the model described the data adequately.

![Figure 36 Kinetic curves of different cooked pastas](image)
According to the Table 23 the rate constant (k), which characterises the velocity of hydrolysis, did not seem to be significantly different among the samples. According to ANOVA tests the type of flour did not have significant effect on this parameter. Moreover it was not significantly lower in RS-enriched pasta products compared to the control products (according to Dunnett’s test). Among the *T. aestivum* pastas, only AA20 had a significantly lower k value compared to the control pasta. Among the *T. durum* products neither of the RS-added products was significantly different from the control pasta according to the Dunnett’s test. ANOVA confirmed that RS-s did not have significant effects on this parameter. The other typical parameter is the equilibrium concentration, C\text{max}. The wheat flour used in the pasta samples had significant effect on the C\text{max} value (ANOVA). Among the *T. aestivum* pastas, the addition of RS did not cause significant difference compared to the control pasta (Dunnett’s test). Among the *T. durum* pastas only DA10 and DA20 samples had significantly lower C\text{max} values compared to the control. From the analysis of the kinetic parameters, RS additives were not shown to have significant effects on the *in vitro* digestion properties of pasta products.

The type of flour seemed to have significant effect on the AUC values (ANOVA). RS-s caused significant changes in the area especially among *T. aestivum* pastas. All of the RS-containing *T. aestivum* pastas had smaller AUC values compared to the control pasta.

### Table 23 Kinetic parameters and digestibility ratio of different pasta products

<table>
<thead>
<tr>
<th>Pasta</th>
<th>C\text{max} (mg/g)</th>
<th>k (1/min)</th>
<th>AUC (t=180 min)</th>
<th>Relative digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>19.26±0.42 ac</td>
<td>0.025±0.002 a</td>
<td>2675.33±23.34 a</td>
<td>100.00</td>
</tr>
<tr>
<td>AA10</td>
<td>19.65±0.44 ac</td>
<td>0.018±0.001 a</td>
<td>2476.67±5.78 bce</td>
<td>92.56</td>
</tr>
<tr>
<td>AB10</td>
<td>19.15±0.40 ac</td>
<td>0.020±0.001 ab</td>
<td>2496.67±7.69 bbev</td>
<td>93.38</td>
</tr>
<tr>
<td>AC10</td>
<td>19.55±0.46 ac</td>
<td>0.018±0.001 b</td>
<td>2451.00±28.29 bde</td>
<td>91.59</td>
</tr>
<tr>
<td>AA20</td>
<td>19.54±0.78 ac</td>
<td>0.015±0.001 b</td>
<td>2262.67±33.75 cdi</td>
<td>84.56</td>
</tr>
<tr>
<td>AB20</td>
<td>18.62±0.47 ac</td>
<td>0.019±0.001 ab</td>
<td>2369.00±19.86 cdi</td>
<td>88.52</td>
</tr>
<tr>
<td>AC20</td>
<td>18.61±0.32 ac</td>
<td>0.020±0.001 ab</td>
<td>2406.33±28.29 cde</td>
<td>89.95</td>
</tr>
<tr>
<td>D control</td>
<td>18.21±0.30 abc</td>
<td>0.024±0.001 ab</td>
<td>2503.67±24.13 ber</td>
<td>100.00</td>
</tr>
<tr>
<td>DA10</td>
<td>16.56±0.41 c</td>
<td>0.025±0.002 ab</td>
<td>2305.67±47.76 cdg</td>
<td>92.05</td>
</tr>
<tr>
<td>DB10</td>
<td>18.12±0.39 bde</td>
<td>0.024±0.002 ab</td>
<td>2505.33±2.33 fh</td>
<td>100.00</td>
</tr>
<tr>
<td>DC10</td>
<td>17.89±0.31 bde</td>
<td>0.022±0.001 ab</td>
<td>2410.33±16.83 dg</td>
<td>96.25</td>
</tr>
<tr>
<td>DA20</td>
<td>17.30±0.28 cd</td>
<td>0.022±0.001 b</td>
<td>2314.67±32.67 cdg</td>
<td>92.45</td>
</tr>
<tr>
<td>DB20</td>
<td>18.62±0.58 cde</td>
<td>0.019±0.002 ab</td>
<td>2409.00±53.52 fij</td>
<td>96.20</td>
</tr>
<tr>
<td>DC20</td>
<td>18.16±0.39 bde</td>
<td>0.020±0.001 ab</td>
<td>2379.33±22.30 dd</td>
<td>95.00</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same column, the values with the same superscript (a-i) are not significantly different (p<0.05)

*represents values significantly different from the control pasta (Dunnett’s test)

†represents ratio of AUC\text{RS enrich. pasta}/AUC\text{control pasta}
Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta)
Tímea Gelencsér, Budapest University of Technology and Economics 2009

(ANOVA, Dunnett’s test). This demonstrates that pastas, enriched with RS-s, can lead to lower glucose liberation under *in vitro* conditions. Investigating the cooking properties of pastas, it was found that the inclusion of RS-s did not affect the breakdown of the starch-protein continuum. Therefore it can be stated that the flour can be partly replaced by starches without the changes of cooking quality and so pastas of moderate glucose release can be developed. Among the *T. durum* samples, DA10, DA20, DC10 and DC20 had significantly lower AUC values compared to the respective control product. The other pastas showed similar changes compared to the *T. durum* control sample. The relative digestibility, referring to the decrease in digestibility, can be calculated as the ratio of \( \text{AUC}_{ \text{RS enriched pasta} } / \text{AUC}_{ \text{control pasta} } \). The relative digestibility of pasta products (using the mean values of the AUC parameters) are also given in Table 23. The RS-enriched pastas seemed to have lower relative digestibility ratios than the control pasta among the *T. aestivum* products. The rate of this relative digestibility declined markedly with the RS addition. Part of this decrease in digestibility may be due to the reduction of the content of fast digestible starch within the products. On the other hand the lipid component from the egg additive can also influence the digestibility of starch in the pasta products according to Crowe et al (2000). RS-rich ingredients caused an 8 to 9 % decrease in digestibility at the 10 % replacement level and a 12 to 15 % decrease when flour was replaced at 20 % level.

The added RS-s had only a small effect on the digestibility of the *T. durum* products. The relative digestibility was reduced approximately by 8 % in the case of Hi-maize 260. The other two starches did not seem to affect the relative digestibility.

Results indicate that not only product composition (*T. aestivum* flour, *T. durum* semolina, egg powder) but also the pasta structure and interactions between RS and other flour components can influence the enzymatic availability of polysaccharides.

### 3.4.4.4 Changes in the RS content due to extrusion and cooking processes

Table 24 summarizes the result of RS content changes in samples from the raw mixtures to the cooked pastas.
Table 24 Resistant starch content of pasta sample before and after processing

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mixture</th>
<th>Extruded sample</th>
<th>Cooked pasta</th>
</tr>
</thead>
<tbody>
<tr>
<td>A control</td>
<td>14.98±0.55 a</td>
<td>15.51±0.41 a</td>
<td>4.47±0.63 a</td>
</tr>
<tr>
<td>AA10</td>
<td>14.67±1.24 a</td>
<td>15.27±0.62 a</td>
<td>4.93±0.12 a</td>
</tr>
<tr>
<td>AA20</td>
<td>16.04±1.29 b</td>
<td>15.12±0.25 a</td>
<td>7.78±0.92 d</td>
</tr>
<tr>
<td>AB10</td>
<td>15.59±0.35 b</td>
<td>15.35±0.32 a</td>
<td>4.66±0.16 a</td>
</tr>
<tr>
<td>AB20</td>
<td>14.57±0.25 a</td>
<td>12.80±0.91 b</td>
<td>5.43±0.55 b</td>
</tr>
<tr>
<td>AC10</td>
<td>15.24±0.74 b</td>
<td>15.16±0.72 a</td>
<td>4.17±0.17 a</td>
</tr>
<tr>
<td>AC20</td>
<td>14.71±0.70 a</td>
<td>15.73±0.96 a</td>
<td>4.84±0.16 a</td>
</tr>
<tr>
<td>D control</td>
<td>12.03±0.94 c</td>
<td>11.31±1.71 b</td>
<td>5.86±0.40 b</td>
</tr>
<tr>
<td>DA10</td>
<td>13.15±0.18 d</td>
<td>13.38±0.14 c</td>
<td>6.87±0.80 c</td>
</tr>
<tr>
<td>DA20</td>
<td>12.31±0.54 c</td>
<td>12.95±0.81 b</td>
<td>6.19±0.81 c</td>
</tr>
<tr>
<td>DB10</td>
<td>13.66±0.46 d</td>
<td>14.04±0.40 c</td>
<td>5.92±0.28 b</td>
</tr>
<tr>
<td>DB20</td>
<td>12.45±1.58 ed</td>
<td>9.02±0.41 d</td>
<td>6.58±0.41 c</td>
</tr>
<tr>
<td>DC10</td>
<td>12.25±0.12 c</td>
<td>12.85±0.24 b</td>
<td>5.59±0.25 b</td>
</tr>
<tr>
<td>DC20</td>
<td>12.93±0.45 c</td>
<td>12.81±0.22 b</td>
<td>5.67±0.16 b</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same column, the values with the same superscript are not significantly different (p<0.05)

The raw samples had quite high RS content before treatments mainly due to the original amylose content of the wheat flours and the RS addition in the case of RS enriched products. Substitution of flour by 20% of RS resulted in only very small increase in the RS content due to the starch fractions of RS additives with higher digestibility. Hence the RS products are not totally resistant against the amylolytic enzymes but contain a higher portion of indigestible starch (Gelencsér et al., 2008). In the case of T. durum raw samples significantly lower RS content was measured compared to the T. aestivum ones indicating the differences between the flours used in the pastas.

Results showed that the extrusion step did not cause significant decrease in the RS content; the cooking, however, reduced it significantly (p<0.05). Based on the results it has been observed that the RS-s used in the preparations are greatly heat sensitive and that cooking is the critical step of the pasta preparing. Although, it should be noted, that the RS content of the RS enriched products was higher after the cooking compared to the control samples respectively, indicating a higher dietary fibre content due to the additives. The pasta with the highest RS content (AA20) as well as the T. aestivum control were chosen for further experiments: in vitro and in vivo GI tests.
3.5 **In vitro GI test of the selected RS enriched pasta**

The digestion curves of the tested materials were analysed as the solution of first-order kinetics, according to Goñi et al. (1997). The nonlinear equation used for the evaluations was the following

\[ C = C_{\text{max}} \left(1 - e^{-kt}\right) \]

where \( C \) is the concentration at \( t \) time, \( C_{\text{max}} \) is the equilibrium concentration, \( k \) is the rate constant and \( t \) is the sampling time. The \( R^2 \) values of the fitted curves were above 0.95 in all cases which could prove the model. Next to \( C_{\text{max}} \) and \( k \), the third characteristic marking the kinetics, was the area under the curve (AUC) referring to the total glucose release (digestibility) over the whole period of time (\( t=180 \) min). The AUC (\( t=120 \) min) has also been calculated to be able to predict the *in vitro* GI of samples. The results of the *in vitro* studies are shown in **Figure 37**.

![Figure 37 Prediction of the *in vitro* GI](image)

The differences among the samples could be obviously seen in the figure and also in **Table 25**. According to our expectations after Goñi et al., 1997 the highest digestibility occurred in the case of bread sample showing a very fast glucose release in the first 30 minutes. After this short period of time the glucose concentration reached the maximum level and there were not any changes in it afterwards. The \( C_{\text{max}} \) and \( k \) values of the pasta sample were not influenced by the RS addition, the AUC on the same time was significantly different (p<0.05) for the two pasta products indicating the effect of RS on the digestibility itself. The GI was predicted
according to Germaine et al. (2008). First the hydrolysis indices were calculated as follows HI=(AUC test food/ average AUC white bread)×100 and the GI\textsubscript{HI} was predicted as follows GI\textsubscript{HI}=39.71+(0.549×HI). Table 25 shows all of the parameters calculated in the \textit{in vitro} GI studies.

**Table 25** Kinetic parameters of the tested samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pasta</th>
<th>Bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS content (g/100g)</td>
<td>Control 4.5±0.6(^a)</td>
<td>RS added 7.8±0.9(^b)</td>
</tr>
<tr>
<td></td>
<td>C(_{\text{max}}) (mg glucose/g sample)</td>
<td>12.27±0.36(^a)</td>
</tr>
<tr>
<td></td>
<td>0.031±0.002(^a)</td>
<td>0.025±0.002(^b)</td>
</tr>
<tr>
<td>AUC (t=180 min)</td>
<td>1824.33±28.92(^a)</td>
<td>1696.33±22.21(^a)</td>
</tr>
<tr>
<td>AUC (t=120 min)</td>
<td>1079.00±13.79(^a)</td>
<td>986.57±10.77(^b)</td>
</tr>
<tr>
<td>Calculated HI</td>
<td>77.91±1.72(^a)</td>
<td>71.23±1.35(^b)</td>
</tr>
<tr>
<td>\textit{In vitro} GI\textsubscript{HI}</td>
<td>82.48±0.95(^a)</td>
<td>78.81±0.74(^b)</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM. Within the same rows, the values with the same superscript are not significantly different (p<0.05)

*bread as reference has the HI of 100

The product containing resistant starch had significantly lower HI (71.23±1.35) compared to the pasta without resistant starch (77.90±1.73). The GI\textsubscript{HI} of the samples differed also significantly, however the difference was small between the two pasta samples (82.48±0.95 and 78.81±0.74 for the control and the RS pasta respectively). These \textit{in vitro} results could prove the effect of RS on the liberated glucose level from pasta and accordingly a significant effect could have been expected also under \textit{in vivo} circumstances. Table 25 shows significant difference between the RS content of the control and RS added pasta. The measurement of RS in the products proved the higher RS content (higher dietary fibre content as well) due to the high-amylose starch applied in the sample.
3.6 *In vivo* GI test of the selected RS enriched pasta

The GI measurement of the selected pasta sample (pasta with 20 % Hi-maize260) was carried out at the University of Maastricht (the Netherlands) following the standard methodology (10 persons, bread as reference) and following strict instructions and protocol (detailed in MATERIALS and METHODS). The measured glycaemic responses after the consumption of the control or RS enriched pasta as well as the reference bread are summarized in the supplement. The average glycaemic responses are shown in Figure 38.

![Figure 38](image)

**Figure 38** Average glycaemic responses after the consumption of the foodstuffs

The area under the curve of the glycaemic response can be calculated on several ways (Brouns et al., 2005). The standard methodology suggests using the *incremental AUC* which is the area over the baseline under the curve, ignoring area beneath the baseline. The baseline is to be determined as the measured glucose level in the 0 min (the first measured data before eating).

According to Figure 38 the curves of the different foodstuffs are obviously different. The highest glucose level occurs in the case of bread consumption and the lowest for the RS enriched pasta. After reaching the maximum glucose level, the values show a slow decrease in the case of RS pasta while the rise of the curve is steeper for the control pasta and the steepest for the bread sample.
This observation can confirm the differences in the digestibility of pasta and bread products. According to (Åkerberg et al., 1998) bread is rapidly digested leading to high blood sugar concentration (high GI) and the fall of the glucose level is quite sheer while sugars are progressively liberated from pasta during digestion, leading to a standard increase in postprandial blood glucose and insulin response as well as a slow decline of the blood sugar level (Tudorică et al., 2002). Therefore pasta is regarded as a product with low glycaemic index (Björck et al., 2000). The Figure 39 showing the changes in the blood glucose level after eating (calculated as the differences between the glucose levels in two subsequent measuring points) can also support this statement.

![Figure 39 Changes in the blood sugar level after the consumption of the investigated foodstuffs](image)

The changes in the blood sugar level are moderate after pasta consumption compared to the reference bread. For the RS enriched and the control pasta the maximum deflection from the baseline is (+) 0.99 mgL⁻¹ and (+) 1.06 mgL⁻¹ respectively while the minimum is (-) 0.57 mgL⁻¹ for both pasta samples. This values are (+) 1.18 mgL⁻¹ and (-) 1.16 mgL⁻¹ for the bread; indicating the more significant effect of bread consumption on the blood sugar level.
The calculated incremental AUC-s based on the data of the individuals (Supplement, page 114) are presented in Table 26.

Table 26 *in vivo* parameters of pasta products

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pasta Control</th>
<th>Pasta RS added</th>
<th>Bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incremental AUC</td>
<td>88.54±91.87 a</td>
<td>67.97±63.60 b</td>
<td>109.87±80.50 a</td>
</tr>
<tr>
<td><em>in vivo</em> GI</td>
<td>72.08±29.95 a</td>
<td>62.11±35.82 a</td>
<td>100*</td>
</tr>
</tbody>
</table>

Values represent means of triplicates ±SEM.

Within the same rows, the values with the same superscript are not significantly different (p<0.05)

*bread as reference has the *in vivo* GI of 100

The results showed big differences among the food samples. The average incremental AUC of the RS added pasta was significantly (p<0.05) smaller compared to the control pasta and the bread. According to the big variance of the individuals the values varied in a wide range from 7.94 to 218.51.

The calculated AUC was not significantly different for the control pasta and the reference bread; however the average value was notably smaller in the case of pasta.

From the incremental AUC the glycaemic indexes can be predicted as the ratio of f/r, where f is an individual subject’s incremental AUC after consuming the test food and r is the incremental AUC for the same subject after consuming the reference food in our case bread.

The GI values measured *in vivo* for the two tested pasta products are shown in Table 26. There was no significant difference between the tested samples according to T-tests, the average GI of the RS pasta at the same time was lower compared to the control one. The remarkable variance however could be improved with the increased number of the volunteers (10 subjects can not represent a population).

The ratio of the GI of RS added pasta and control pasta for the individuals are shown in Figure 40.
Among the subjects there were 5 whose GI was lower for RS added pasta compared to the control sample, in two cases the GI-s were not significantly different between the two products and three volunteers showed higher GI for RS pasta. The difference among the subjects highlights the variability of the individual’s metabolism indicating a larger group of people tested when GI is to be predicted.

Taken all round it can be stated that the selected resistant starch (Hi-maize260) is proper as a flour replacer in food applications and can lead to a reduced GI of the products. Additionally the pasta containing resistant starch may be an appropriate basis in the diet of individuals with overweight and obesity.

**Figure 40** The ratio of $\text{GI}_{\text{RS pasta}}$ and $\text{GI}_{\text{control pasta}}$ for the single individuals
Summary of the results with pasta products reveal the next important facts:

1, the rheological properties of pasta samples are greatly affected by the RS addition; the inability of resistant starches to gelatinize cause a significant decrease in the viscosity parameters of the RVA curves.

2, according to the results of cooking tests, it can be concluded that RS-s do not affect the characteristics of pasta products during the cooking procedure significantly in spite of the expectations after the RVA measurements.

3, the digestibility studies proved that the resistant starch- added mixtures reached smaller AUC values in each case compared to the control sample. The differences were statistically confirmed, thus it follows from this that the resistant starches caused a significant (p<0.05) reduction in the absolute glucose release (digestibility) but did not have any effects on the shape and characteristics of kinetics in the case of raw mixtures.

4, by evaluating the changes after extrusion our conclusion could be that this technological process caused alterations in the digestibility of all products this alteration however was not significant in all cases. The extent and type of changes were dependent on the flour quality used in the pasta as well as the properties of resistant starches added into the products.

5, cooking procedure is the determinative step in the pasta process causing the radical increase of the digestibility and the decrease of the RS content of the pasta samples. Based on the results it has been observed that the RS-s used in the preparations are greatly heat sensitive and that cooking is the critical step of the pasta preparing. Although, it should be noted, that the RS content of the RS-enriched products is higher after the cooking compared to the control samples respectively, indicating a higher fibre content due to the additives. The pasta with the highest RS content (AA20) as well as the T. aestivum control was chosen for further in vitro and in vivo GI experiments.

6, the sensory properties of the RS enriched products and control samples are not significantly different according to the test panel. It follows from this that resistant starches can be used in pasta products without the changes of the sensory properties.
7, according to the *in vitro* GI tests RS can have great impact (reducing effect) on the glucose response simulated in tubes indicating similar effects also under *in vivo* circumstances. The *in vitro* GI values are significantly different for the two tested pasta products.

8, the *in vivo* GI values for the two selected pasta products are not significantly different, the average GI of the RS pasta at the same time is lower compared to the control one. The remarkable variance could be improved with the increased number of the subjects.

All in all it can be stated that resistant starches are appropriate additives in the starch based products to enhance the dietary fibre content of foodstuffs. The food preparation steps however have to be taken into account due to the proved and variable heat sensitivity of the starches and the effects on the GI.
SUMMARY and NEW SCIENTIFIC RESULTS

In my PhD studies I have investigated the properties of resistant starches in raw state, after heat load and in real food materials. Resistant starches play significant role in the human diet therefore the knowledge about their characteristics are remarkably important. In my studies I could prove that the different type of resistant starches behave totally different compared to native starches observed in rheological and digestibility tests. I confirmed that

1, resistant starches are not able to gelatinize in the applied RVA procedure, consequently the viscosity parameters of the mixtures prepared by RS addition decrease significantly with the amount of RS (diluting effect). The texture of the products prepared with RS-s might be notably influenced by the rheological properties of resistant starches.

2, the water absorption (WA) values of resistant starches are very variable according to the type and origin of RS; the WA values have to be calculated and considered when a product is to be developed.

3, the enzymatic digestibility of different RS-s varies according to their origin and type (RS2, RS3, and RS4). Lower resistance can be observed in the case of RS3 starches.

I have also studied the effects of different heat treatments on the properties of RS and proved that

1, the native starches are sensitive enough to show changes in the RVA due to the dry heat load while the RS-s are not influenced by the treatment and they do not gelatinize in the applied procedure. The inability of resistant starches to gelatinize may lead to decreased viscosity and inconvenient textural properties of foodstuffs enriched with RS. This statement has to be taken into account in the food development and additionally the use of resistant starches must be limited and well calculated to get products with efficient properties and good consumer value.

2, the WA values are not significantly influenced by the heat load however small changes occurred in the case of all starches leading to increased viscosity values of the native starches.

3, the enzymatic digestibility of the starches is strongly influenced by the heat load. The dry heat treatment cause significant, but definitely smaller effects than the cooking. Cooking
seems to be the most determinative process influencing the availability of starches to amylases.

**The application of resistant starches in bread products resulted in that**

1. the use of resistant starches can negatively influence the physical properties of bread samples mainly the volume of the products. The RS addition cause inhomogeneous, wet crumb structure containing starch clusters deteriorating the consumer value of the products.
2. the sensory properties of the samples are not significantly different according to the control panel, the taste of the RS added products however is less preferred.
3. the RS-s do not affect the digestibility of dough and bread samples significantly, the RS content of the samples however is radically reduced by the baking.
4. according to the changes in the RS content of samples it can be concluded that it is difficult to use RS in bread samples. The resistant starches show namely strong heat sensitivity and loose their resistance during the baking process. The baking at the same time can lead to the appearance of retrograded starch indicating higher RS content after a short storage.

**The application of RS in pasta products reflected that**

1. the rheological properties of pasta samples are greatly affected by the RS addition; the inability of resistant starches to gelatinize cause a significant decrease in the viscosity parameters of the RVA curves.
2. according to the results of cooking tests, it can be concluded that RS-s did not affect the characteristics of pasta products during the cooking procedure significantly in spite of the expectations after the RVA measurements.
3. in the digestibility studies the resistant starches caused a significant (p<0.05) reduction in the absolute glucose release but did not have any effects on the shape and characteristics of kinetics in the case of raw mixtures.
4. the technological process (extrusion) caused alterations in the digestibility of all products. The extent and type of changes were dependent on the flour quality used in the pasta as well as the properties of resistant starches added into the products.
5, cooking procedure is the determinative step in the pasta process causing the radical increase of the digestibility and the decrease of the RS content of the pasta samples. Based on the results it has been observed that the RS-s used in the preparations are greatly heat sensitive and that cooking is the critical step of the pasta preparing.

6, the sensory properties of the RS enriched products and control samples were not significantly different according to the test panel. It follows from this that resistant starches can be used in pasta products without the changes of the consumer values.

7, according to the \textit{in vitro} GI tests RS can have great impact (reducing effect) on the glucose response simulated in tubes indicating similar effects also under \textit{in vivo} circumstances.

8, the \textit{in vivo} GI values for the two tested pasta products were not significantly different, the average GI of the RS pasta at the same time was lower compared to the control one. It can be concluded that RS pasta may have lower digestibility but a large number of subjects is needed to prove this statement.

Summarizing all of the results the main conclusion can be that resistant starches are good flour replacers in products, their rheological properties, however, have to be taken into account to have products with proper texture. The food processing steps using heat load cause significant decrease in the amount of RS therefore their effects have to be limited. RS has notable nutritional role proved by our studies, its use in starch-based products thus can be a great help to increase the fibre content, decrease the digestibility of products and to prepare foods for obese and overweight individuals.
REFERENCES


Applications manual for the RVA (1998) Newport Scientific Pty. Ltd., Australia


Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta)
Tímea Gelencsér, Budapest University of Technology and Economics 2009


Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta)
Tímea Gelencsér, Budapest University of Technology and Economics 2009

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SUPPLEMENT

1. RVA curves of different native starches after head load

### Rice starch

<table>
<thead>
<tr>
<th></th>
<th>PV (cP)</th>
<th>TR (cP)</th>
<th>BD (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
<th>PT (min)</th>
<th>PTp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2505.0</td>
<td>2109.5</td>
<td>395.5</td>
<td>3355.5</td>
<td>1246.0</td>
<td>6.5</td>
<td>79.4</td>
</tr>
<tr>
<td>10min</td>
<td>2729.5</td>
<td>2331.5</td>
<td>398.0</td>
<td>3572.0</td>
<td>1240.5</td>
<td>6.6</td>
<td>79.9</td>
</tr>
<tr>
<td>20min</td>
<td>2809.5</td>
<td>2352.5</td>
<td>457.0</td>
<td>3696.0</td>
<td>1343.5</td>
<td>6.5</td>
<td>79.9</td>
</tr>
<tr>
<td>30min</td>
<td>2857.0</td>
<td>2395.0</td>
<td>462.0</td>
<td>3751.0</td>
<td>1356.0</td>
<td>6.5</td>
<td>79.8</td>
</tr>
</tbody>
</table>

### Maize starch

<table>
<thead>
<tr>
<th></th>
<th>PV (cP)</th>
<th>TR (cP)</th>
<th>BD (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
<th>PT (min)</th>
<th>PTp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2962.0</td>
<td>1959.5</td>
<td>1002.5</td>
<td>3133.0</td>
<td>1173.5</td>
<td>5.1</td>
<td>76.3</td>
</tr>
<tr>
<td>10min</td>
<td>3307.0</td>
<td>2170.5</td>
<td>1136.5</td>
<td>3387.0</td>
<td>1216.5</td>
<td>5.1</td>
<td>75.9</td>
</tr>
<tr>
<td>20min</td>
<td>3379.0</td>
<td>2182.5</td>
<td>1196.5</td>
<td>3516.0</td>
<td>1333.5</td>
<td>5.0</td>
<td>75.1</td>
</tr>
<tr>
<td>30min</td>
<td>3442.5</td>
<td>2194.0</td>
<td>1248.5</td>
<td>3559.5</td>
<td>1365.5</td>
<td>5.0</td>
<td>75.5</td>
</tr>
</tbody>
</table>
2. Glycaemic responses of the subjects in the *in vivo* studies
KÖSZÖNETNYILVÁNÍTÁS

Disszertációim befejezéseként köszönetet szeretnék mondani mindenkinnek, aki segítségével, emberi támogatásával hozzájárult munkám eredményes elvégzéséhez.

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