BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL AND BIOENGINEERING
OLÁH GYÖRGY Ph.D SCHOOL

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

Thesis book

Author: ***Tímea Gelencsér***
M.Sc. in Bioengineering

Supervisor: ***András Salgó***
Full Professor

Department of Applied Biotechnology and Food Science

Budapest · 2009

1. INTRODUCTION AND AIMS

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; 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. 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 dietary 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. RS is classified into four different types according to its resistance. **RS1** is physically inaccessible starch, such as those found in partially milled grains, seeds, legumes and tubers. **RS2** means native starch granules that are protected by the conformation or structure of the starch granules. Raw potato, green banana, and high-amylose maize starches are the sources of this RS2 type. **RS3** is the result of repeated heating and cooling of starch, meaning retrograded starch (mainly amylose), occurring in cooked and cooled potatoes, and ready-to-eat breakfast cereals. **RS4** type is

chemically modified starch which includes hydroxypropyle starches, acetate starches, phosphate starches, and citrate starches RS4.

Bread and pasta products belong to the basic foodstuffs having an important role in the human consumption. 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 causing fast glucose release and absorption 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 goal was to investigate the properties of different resistant starches and to use them in starch-based products as dietary fibre sources.

The aims of my PhD studies were the follows:

1, to investigate the physicochemical and digestibility properties of resistant starches of different origin 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

Three native starches (maize, wheat and rice), and six resistant starches were studied (Hi-maize 260 and Hi-maize 1043 as RS2, Novelose 330, Crystalean and C*Actistar as RS3 as well as Fibersym 70 as RS4 samples) as is and in their stoichiometric mixtures. The effects of RS on the mixtures and the main differences among native and resistant starches were investigated using rapid visco analyzer (RVA), water absorption and in vitro digestibility methods. After the measurements in raw form, starches were heat treated (dry heat load and moisture heat treatment) to evaluate their sensitivity on food processing. The RVA parameters, the water absorption and the digestibility of the samples were recorded. The amylolitic hydrolysis was carried out using α-amylase and the liberated glucose level which relates to the available starch content, was measured.

In the next step three RS-s were selected (with the best properties according to the heat load tests: Hi-maize 260, Hi-maize 1043 and Fibersym 70) to use them in starch-based products.

Bread samples were baked according to the Hungarian Standard methodology from BL-55 flour using RS-s in 20 % (w/w) as flour replacer. The products were tested from physical and sensory properties as well as in vitro digestibility point of view. The RS content of samples was also tested before and after the baking procedure using the standard AACC/AOAC method.

Pasta samples were prepared as short cut pasta from *T. aestivum* flour and *T. durum* semolina using the RS-s in 10 and 20 % (w/w) as flour replacer. Pastas were tested according to their

physical, rheological and sensory properties as well as their digestibility. The digestibility of the raw mixtures, extruded and cooked samples was measured to predict the effects of food processing steps on the nutritional properties of the pasta samples. The RS content before and after cooking was also evaluated by the standard AACC/AOAC method. The pasta with the best sensory and digestibility properties was selected for further *in vitro* and *in vivo* GI tests. The *in vitro* GI tests were carried out using a multi-enzyme procedure to simulate the human digestion system. The *in vivo* GI tests were carried out following the standard methodology with 10 healthy subjects using bread as reference keeping strict instructions.

3. NEW SCIENTIFIC RESULTS

3.1. Comparison of native and resistant starches

The investigations of pure starches showed that resistant starches are not able to gelatinize in the applied RVA procedure, consequently the viscosity parameters of the mixtures 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. 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. The enzymatic digestibility of different RS-s varies according to their origin and type (RS2, RS3, and RS4) as well, indicating the use of RS2 samples in food preparation.

The detailed investigations of starch mixtures proved that the origin and type of starches have significant effect on the nutritional properties of samples. The resistant starches have remarkably different properties compared to native starches. This study was the first which used the RVA procedure and the digestibility tests for the comparison of the physical and functional properties of starches differing in nutritional quality. It has been proved that RS components cause significant changes in the characteristics of native-resistant starch mixtures.

3.2. Investigations of the effects of heat load on the properties of starches

Different heat treatments on starches confirmed that native starches were sensitive enough to show changes in the RVA due to the dry heat load while the RS-s were not influenced by the treatment and they did 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. 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. 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 effects of heat load on the RVA parameters and digestibility of starches were simulated. The heat sensitivity of starches was proved by using two different heat treatment procedures. The digestibility of starches was significantly increased after the heat load indicating the great effects of food processing on the availability of starches to amylases.

3.3. The use of RS in bread products

The use of resistant starches in bread can negatively influence the physical properties mainly the volume of the products. The RS addition cause inhomogeneous, wet crumb structure containing starch clusters deteriorating the consumer value of the products. The sensory properties of the samples are not significantly different; the taste of the RS added products however is less preferred than the taste of the control sample. The RS-s did not affect the digestibility of dough and bread samples significantly; the RS content of the samples however is radically reduced by the baking. The changes in the RS content of samples highlight the difficulties of the use of RS and its protection in bread samples. The resistant starches show namely strong heat sensitivity and loose their resistance during the baking process.

3.4. The use of RS in pasta products

The rheological properties of pasta samples are greatly affected by the RS addition. The results of cooking tests confirmed 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. In the digestibility studies 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. 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 is not significant in all cases. The extent and type of changes are dependent on the flour quality used in the pasta as well as the properties of resistant starches added into the products. 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. The RS-s used in the preparations are greatly heat sensitive and cooking is the critical step of the pasta preparing.

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 value of product.

The in vitro and in vivo GI of pasta samples were detected and results proved the impact of RS on the nutritional quality of samples. The in vitro GI prediction resulted in significant difference between the control and the RS-added samples.

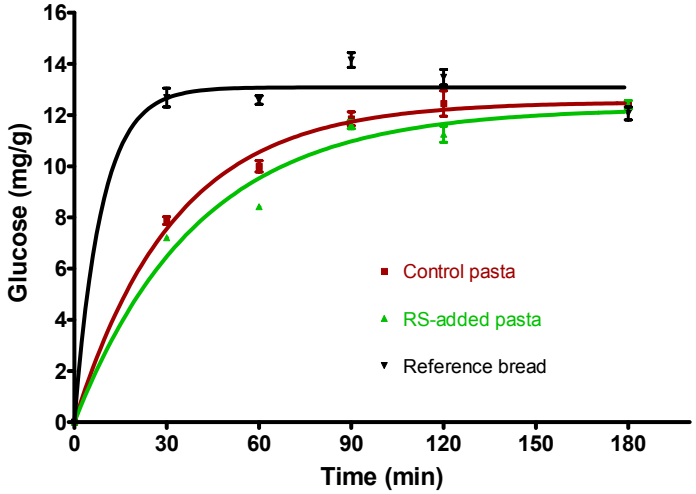

Figure 1. Kinetic curves of in vitro GI tests

The in vivo GI measurements showed a big variance among the subjects causing not significant difference between the two pasta samples.

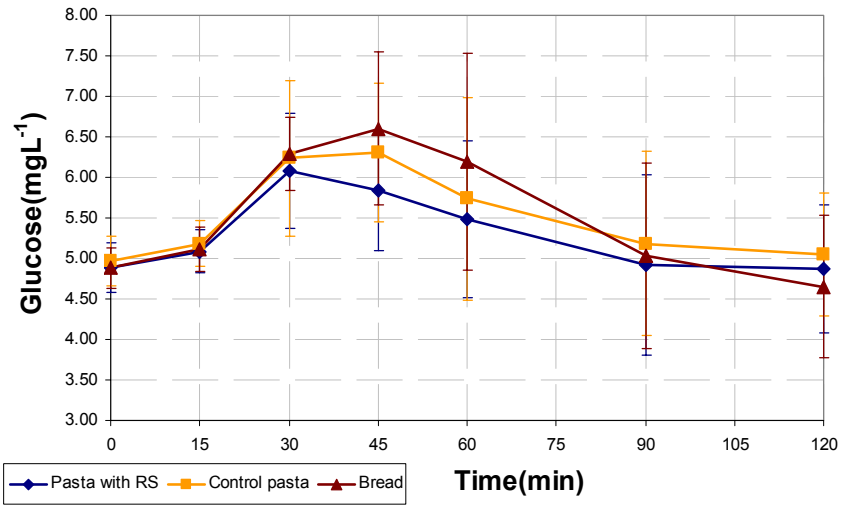

Figure 2. Results of in vivo GI tests

4. THESES

I. The investigations of pure starches showed that resistant starches are not able to gelatinize in the RVA procedure. The water absorption (WA) values and the enzymatic digestibility of resistant starches are very variable according to the type and origin of RS. Using starch mixtures I have proved that the resistant starches can be used in products but only in limited amount. This study was the first which used the RVA procedure and the digestibility tests for the comparison of the physical and functional properties of starches differing in nutritional quality (**1**).

II. The use of resistant starches in bread can negatively influence the physical and sensory properties of the products. I have proved thet the RS content of the samples is radically reduced by the baking indicating their heat sensitivity. The changes in the RS content of samples highlight the difficulties of the use of RS and its protection in bread samples (**16**).

III. The rheological properties of pasta samples are greatly affected by the RS addition, the cooking parameters at the same time are not influenced by the RS components. In the digestibility studies 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. The applied pasta processing (extrusion, cooking) cause changes in

the digestibility of the samples, this changes are significant after cooking. The RS-s used in the samples show high heat resistance on the cooking step (**3**).

IV. The sensory properties of the RS enriched products and control samples are not significantly different according to the test panel, indicating the use of RS in pasta products. This study was the first which used a large number of samples in the monitoring of the effects of different resistant starches on the physical, rheological and sensory properties of pasta (**2**).

V. The in vitro GI tests proved the functional effects of high-amylose starch. According to the in vitro tests in can be concluded that the applied RS has a large decreasing effect on the digestibility of pasta. In the in vivo tests I have proved the differences in the metabolism and biological responses of subjects after the consumption of RS enriched product (**5**).

5. PRACTICAL APPLICATION OF THE RESULTS

The results of my studies on resistant starches highlight the importance of the application of RS-s as fibre sources in starch-based products. The studies on bread and pasta can give a great help to manufacturers in their product development as well as in their analytical tests in the quality control. The theses also highlights the importance of rheological tests and the easy use of RVA when the texture of the product is remarkably important and has to be studied, moreover indicates the use of digestibility tests when the nutritional characteristics are to be predicted. The studies on heat load and the effects of food preparation steps call the attention to optimize the food processing aiming to protect RS components in the products. The results confirm the favourable properties of resistant starch enriched bread and pasta samples indicating their use in the treatment of obesity and overweight.

This study was supported by **DiOGenes** which is the acronym of the project 'Diet, Obesity and Genes' supported by the European Community (Contract No. FOOD-CT-2005-513946). The members of the project are listed on the web-site of the project: http://www.diogenes-eu.org.

6. PUBLICATIONS AND PRESENTATIONS

Article in international journal with impact factor published in English

1. **T. Gelencsér**, R. Juhasz, M. Hódsági, Sz. Gergely and A. Salgó, Comparative study of native and resistant starches, Acta Alimentaria, 37(2):255-270 **2008***, IF: 0.398*

2. **T. Gelencsér**, V. Gál, M. Hódsági, A. Salgó, Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta, *Food and Bioprocess Technology: An International Journal, 1:171-179* **2008***, IF: not known (started in 2008)*

3. **T. Gelencsér,** V. Gál, A. Salgó, Effects of applied process on the in vitro digestibility and resistant starch content of pasta products, *Food and Bioprocess Technology: An International Journal, DOI:* 10.1007/s11947-008-0105-7, **2008***, IF: not known (started in 2008)*

4. A. Smeets, M. Lejeune, **T. Gelencsér**, A. Salgó, M. Westerterp-Plantega, The effects of a meal containing resistant starch on energy expenditure, hormones, and satiety, *submitted to Journal of the American College of Nutrition*, **2008***, IF:2.452*

5. **T. Gelencsér**, A. J. Smeets, M. Lejeune, M. S. Westerterp-Plantega, A. Salgó, Comparison of in vitro and in vivo glycaemic index of pasta prepared with and without resistant starch addition, *submitted to Food Chemistry*, **2008***, IF:2.433*

Abstract in international journal with impact factor published in English

6. A. Smeets, **T. Gelencsér**, A. Salgó, M. Westerterp-Plantega, The accut effects of lunch containing resistant starch on energy and substrate utilization, ghrelin, GLP-1, PYY concentrations and appetite. Appetite, 51:400, **2008***, IF: 1.727*

Article in international journal with impact factor published in English not used in the dissertation

7. Juhász, R., Szilveszter, G., **Gelencsér, T**., Salgó, A. Relationship Between NIR Spectra and RVA Parameters During Wheat Germination, Cereal Chemistry, 82 (5):488-493 **2005***, IF:1.104*

Oral presentation in English

8. **T. Gelencsér**: Investigations of resistant starches. 5th European Young Cereal Scientists and Technologists Workshop (5EYCSTW) (5-7 July, 2006, Gaziantep, Turkey)

9. **T. Gelencsér**: Investigations of resistant starch enriched pasta products.6th European Young Cereal Scientists and Technologists Workshop (6EYCSTW) (28 April -3 Mai, 2007, Montpellier, France)

10. **T. Gelencsér**: Application of resistant starch products in the development of foodstuffs with reduced GI. Diogenes Consortium Meeting & Training (22-24 September, 2008, Prague, Czech Republic)

11. **T. Gelencsér,** V. Gál, M. Hódsági, A. Smeets, M. Lejeune, M. Westerterp-Plantega, A. Salgó: Investigations of pasta products prepared with and without resistant starch addition. International Scientific Conference on Cereals – their products and processing (27-28 October, 2008, Debrecen, Hungary)

12. M. Hódsági, **T. Gelencsér**, A. Salgó: The effects of different baking conditions on the in vitro digestibility of resistant starch enriched bread rolls. International Scientific Conference on Cereals – their products and processing (27-28 October, 2008, Debrecen, Hungary)

Oral presentation in Hungarian

13. **Gelencsér T.:** Rezisztens keményítők tulajdonságainak vizsgálata. Conference of PhD students (BME), Faculty of Chemical and Bioengineering (07 February, 2006, Budapest, Hungary)

14. **Gelencsér T.:** Csökkentett glikémiás indexű termékek fejlesztése és vizsgálata. Conference of PhD students (BME), Faculty of Chemical and Bioengineering (07 February, 2007, Budapest, Hungary)

15. **Gelencsér T.:** Rezisztens keményítővel kiegészített tészta termék glikémiás indexének meghatározása. Conference of PhD students (BME), Faculty of Chemical and Bioengineering (07 February, 2008, Budapest, Hungary)

16. **Gelencsér T.:** Rezisztens keményítővel kiegészített termékek vizsgálata. 330th scientific colloquium of the Central Food Research Institute (07 March, 2008, Budapest, Hungary)

Proceeding of oral presentation in proceedings of international conference in English

17. **T. Gelencsér,** V. Gál, M. Hódsági, A. Smeets, M. Lejeune, M. Westerterp-Plantega, A. Salgó: Investigations of pasta products prepared with and without resistant starch addition. International Scientific Conference on Cereals – their products and processing (27-28 October, 2008, Debrecen, Hungary) Ed by Győri Z., Sipos P., Ungai D. ISBN 978-963-9732-38-4, pp. 79-83. (**2008**)

18. M. Hódsági, **T. Gelencsér**, A. Salgó: The effects of different baking conditions on the in vitro digestibility of resistant starch enriched bread rolls. International Scientific Conference on Cereals – their products and processing (27-28 October, 2008, Debrecen, Hungary) Ed by Győri Z., Sipos P., Ungai D. ISBN 978-963-9732-38-4, pp. 275-281. (**2008**)

Proceeding of poster presentation in proceedings of international conference in English

19. **T. Gelencsér**, V. Gál, A. Salgó: Investigations of resistant starch enriched bread products. 4th International and the 6th Croatian Congress of Cereal Technologists "Flour-Bread '07" (24-27 October, 2007, Opatija, Croatia) Ed by Zaneta Ugarcic-Hardi, Proceedings, ISBN 978-953-7005-15-3, pp. 444-450. (**2007**)

Abstract in book of abstracts of international conference in English

20. **T. Gelencsér,** A. Salgó: Investigations of resistant starches. 5th European Young Cereal Scientists and Technologists Workshop (5EYCSTW) (5-7 July, 2006, Gaziantep, Turkey) p. 7.

21. **T. Gelencsér**: Investigations of resistant starch enriched pasta products.6th European Young Cereal Scientists and Technologists Workshop (6EYCSTW) (28 April -3 Mai, 2007, Montpellier, France) p. 59.

Abstract in book of abstracts of national conference in Hungarian

22. **Gelencsér T.:** Rezisztens keményítővel kiegészített termékek vizsgálata. 330th scientific colloquium of the Central Food Research Institute (07 March, 2008, Budapest, Hungary) p. 9.

Poster presentation in English

23. **T. Gelencsér**, A. Salgó: Cereal based low GI products (pasta and bread), Diogenes Consortium Meeting & Training (2-4 October, 2006, Mallorca, Spain)

24. **T. Gelencsér**, V. Gál, A. Salgó: Investigations of resistant starch enriched bread products. 4th International and the 6th Croatian Congress of Cereal Technologists "Flour-Bread '07" (24-27 October, 2007, Opatija, Croatia)

25. M. Hódsági, **T. Gelencsér,** A. Salgó: Investigations of resistant starches. Conference of PhD students (BME), Faculty of Chemical and Bioengineering (07 February, 2008, Budapest, Hungary)