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## **Recent results of investigations of resistant starches**

### **Thesis book**

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## 1. INTRODUCTION AND AIMS

Recently, eating habits have changed in the developed countries. The easily digestible, high calories containing foods are consumed every day which led to the spread of obesity worldwide. Obesity puts people at risk for hypertension, dyslipidemia, diabetes mellitus type 2, heart disease, and many other chronic disorders. A promising solution can be the consumption of food prepared with dietary fibres that have several health benefits. The resistant starch (RS), which is a type of dietary fibre as well, has been defined 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. The consumption of resistant starches may improve glucose and lipid metabolism and can reduce the risk of the above mentioned disorders.

The resistance can derive from different causes; hence, RSs can be divided into four groups: RS1 is physically inaccessible for digestive enzymes found, *e.g.*, in grains and seeds. RS2 is defined as intact granular starch, for example, raw potato and high amylose starches belong to this type. RS3 mainly represents retrograded starch, *e.g.*, cooked and cooled starches; while RS4 means chemically modified starch made by cross-linking with chemical reagents, for instance, citrate and phosphate starches.

Due to their health benefits, resistant starches are widely investigated with different analytical methods. Generally, the morphological, structural, thermal, rheological properties and digestibility characteristics of starches are enjoying increased attention due to their great applicability in food industry. In case of resistant starches, one of the most important methods is the enzymatic degradability test. Several mono- and multi-enzymatic test methods for detection of starch hydrolysis and RS content are available. The effects of resistant starch addition in native-resistant starch mixtures can be measured by analyzing their rheological properties (Gelencsér, 2009<sup>1</sup>) using rapid visco analyzer (RVA) or farinograph method. Mixolab, a new dough quality testing device, can also determine the rheological, gelatinisation and enzymatic properties of flours. However, there is no information related to its utilization on the effects of resistant starch addition to samples. The structural characteristics of starches can be analyzed using near infrared (NIR) spectroscopy. However,

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<sup>1</sup> Gelencsér (2009) Comparative study of resistant starches and investigations of their application in starch-based products (bread and pasta). PhD Thesis. BUTE.

it has not been studied whether NIR spectra has any information concerning the cause of resistance.

An understanding of the relationship between structural characteristics and functional as well as nutritional properties of starches can be very important for food scientists. Recently, several reports have been carried out to search for correlations between the structural characteristics of starches and their thermal or rheological (RVA) properties. However, there are only few pieces of information about the effects of starch pasting properties on starch digestibility.

It was shown in previous studies that resistant starches are sensitive for the different heat treatments (*e.g.* cooking, baking) used in the food processing which can cause changes especially in the resistance (Gelencsér, 2009). However, the alteration of the technological conditions may improve RS content which indicates the necessity of the investigations of the effects of different conditions. The microwave irradiation, a frequently applied technology nowadays, can be advantageous to the formation of RS; however, the research about its effects on RSs is limited. It is possible to increase the RS3 level of heat-treated starch-based samples due to retrogradation in case of storage and frozen storage. Thus, it is important to investigate the effects of the freezing itself on RS enriched samples.

Commercial resistant starches can yield high quality products (bread, pasta, cookies, pudding, yoghurt etc.) not attainable otherwise with traditional insoluble fibres. However, they can be applied only in limited amount without causing changes in sensory and physical properties of foods.

**The aims of my PhD studies were the follows:**

- 1) to investigate the physicochemical characteristics of resistant starches compared to native starches using near infrared spectroscopy as a new method in RS analysis
- 2) to determine the effects of moisture heat treatment on resistant starch containing mixtures applying a mono-enzymatic three-hour long hydrolysis procedure and to investigate the effects of microwave treatments on the properties of resistant starches compared to native starches

- 3) by using this microwave-treated model system, the sensitivity of two different spectrophotometers is also tested
- 4) to search for correlations between rheological and enzymatic digestibility properties of mixtures containing resistant and native starches
- 5) to investigate the applicability of Mixolab as a new rheological device in the analysis of RS containing starch-based samples
- 6) to analyze the effects of the alterations of different baking conditions on the digestibility of RS containing bread samples and to examine the effects of different baking and freezing technologies on the digestibility, physical and sensory properties of bread samples.

## 2. MATERIALS AND METHODS

Based on the previous research of [Gelencsér \(2009\)](#) which was carried out on resistant starches; two types of resistant starches were selected for my studies: RS2 and RS4 starches. Hi-maize<sup>TM</sup> 260 (National Starch and Chemical GmbH, Hamburg, Germany) was applied as RS2 while Fibersym<sup>TM</sup> 70 or Fibersym<sup>TM</sup> RW (Loryma GmbH, Zwingenberg, Germany) was used as RS4. Additionally, the properties and heat treatment behaviour of two native starches: maize (S4126) and wheat (S5127) starches (Sigma Aldrich Co., St. Louis, MO, USA) were investigated and compared to RSs.

The native and resistant starches were recorded by dispersive NIR spectrometer as is and in their physical mixtures (20 %, 40 %, 60 % and 80 % (w/w)) as well. The whole NIR spectra and the most characteristic absorption bands for carbohydrates were analyzed.

The investigation of the effects of cooking was carried out on the mixtures of native and resistant starches as well using *in vitro* enzymatic digestibility test. The amyolytic hydrolysis was performed using *alpha*-amylase (containing 3 U/mL amyloglucosidase) and the liberated glucose level which relates to the available starch content, was measured. The effects of microwave treatments (according to a 2<sup>2</sup> experimental design; time of the treatment \* power of the treatment = hypothetically transmitted energy of treatment: 9 kJ, 18 kJ, 45 kJ and 90 kJ, respectively) on resistant and native starches were detected using the *in vitro* enzymatic digestibility method, rheological (RVA) and spectroscopic (NIR) methods. Using these

microwave-treated samples, the sensitivity of a dispersive NIR spectrometer and a Fourier transform NIR (FT-NIR) spectrometer was compared to each other.

The results of different *in vitro* enzymatic digestion tests as well as the results of an RVA method of resistant and native starches and their mixtures were used to search for correlation between the different methods (Pearson's correlation).

Using Mixolab device (Chopin+ protocol), the effects of the addition of RS2 (5 %, 10 %, 15 %, 20 % and 25 %) were determined on the rheological properties of commercial bread flour-, *Triticum (T.) durum* pasta flour- and *T. aestivum* pasta flour-based samples.

The effects of alteration of baking conditions (the weight of the bread roll: 55 g, 130 g, 205 g, the temperature of the baking: 180 °C, 210 °C, 240 °C) were tested on the *in vitro* enzymatic digestibility of RSs (RS2, RS4) enriched samples (3<sup>3</sup> experimental design). Besides the *in vitro* enzymatic test, the effects of different freezing technologies (freezing of pre-baked products then full-baking; dough freezing then full-baking; full-baked products from frozen pre-baked bread then freezing) on RS2 containing bread products were analyzed using physical and sensory evaluation methods as well.

### **3. NEW SCIENTIFIC RESULTS**

#### **3.1. Investigation of resistant starches using near infrared spectroscopy**

The investigated characteristic carbohydrate I, II, III and IV regions (1575–1590 nm, the first overtone of O–H stretching; 2080–2130 nm, the combination of O–H bending and C–O stretching; 2275–2290 nm; the combination of O–H stretching and C–C stretching and 2310–2335 nm, the combination of C–H bending and C–H stretching) were sensitive on the properties of RSs in different extent. The vibrations of the regions of the 2080–2130 nm and the 2275–2290 nm provided the most comparable and changeable regions of the samples. Moreover, it can be stated that the addition of high amylose RS2 starch thus the changes of the amylose-amylopectin ratio can be sensitively followed up in carbohydrate II region. The phospho-ester bounds containing RS4 was not so characteristic probably due to reduced mobility of amorphous chains; however, the RS4 addition can be detected. The carbohydrate III region is sensitive for the differences of amylose-amylopectin ratio as well. The addition of

wheat-based RS4 can be differentiated only in maize-based mixtures due to the different botanical origin. The differences of amylose-amylopectin ratio can be observed in this region the most sensitively.

### **3.2. Study of the effects of different heat treatments on resistant and native starches**

The *cooking* affected significantly higher degradability in wheat, maize and RS4 starches compared to RS2 starch. RS4 lost the majority of its enzymatic insusceptibility against *alpha*-amylase while RS2 possibly changed to RS3 and became more resistant in the applied test. In maize- or wheat starch-based mixtures, the addition of RS2 reduced the values of total area under the curve (totally liberated glucose during a three-hour period, *AUC*) as well as the rate constant of the digestion (*k*) linearly. These results seem to be promising and important facts for the application of RS2 in food industry because *k* and *AUC* values can be predicted in the function of high amylose RS2 addition. Additionally, no synergistic effects can be determined in maize- or wheat-based model systems, which is also notable information for product development.

The increasing energy of the *microwave treatment* caused no significant tendencies in the digestibility of resistant starches; the characteristics of the kinetic curves remained unchanged. The resistant starches did not gelatinise even after microwave treatments in the applied RVA procedure. However, applying these starches in 40% of native starch containing mixtures, the changes of RSs are also demonstrable. The microwave treatments weakened the rheological properties of all starches. The NIR spectroscopic study even highlighted the smallest changes in the starch vibrations (2080–2130 nm; 2270–2290 nm) alluding to the structural changes of starches affected by the lowest microwave energy. This study was the first which has investigated the microwave treatments caused effects on the enzymatic, rheological and spectroscopic properties of resistant starches.

### **3.3. The comparison of the sensitivity of two different NIR spectrometers**

The dispersive NIR spectrometer is applicable to follow up the structural changes of starches caused by microwave treatments while the FT-NIR spectrometer showed lower sensitivity despite its higher resolution. The differences between the two spectrometers were the most significant in the carbohydrate III region.

### **3.4. Correlation results between enzymatic and rheological methods**

Significant correlations were detected between two different *in vitro* enzymatic methods as well as RVA method in case of untreated and cooked starches and mixtures of starches which can provide useful information for the prediction of *in vitro* digestibility. Additionally, the changes in RVA parameters can contribute to the change of enzymatic digestibility in raw and cooked samples containing native and resistant starches.

### **3.5. Application of Mixolab in RS analysis**

The Mixolab is applicable to follow up the RS addition (quality decreasing effect) in bread, *T. durum* and *T. aestivum* pasta flour-based samples. Depending on the medium, the parameters of the gelatinisation, breakdown or retrogradation phases are determining. The *C5* parameter, which is the maximum torque after cooling, can be applied for the prediction of RS addition to flours independently on the medium. This study was the first, which has applied the Mixolab technology for the detection of RS addition to different flours.

### **3.6. Investigation of the effects of the alterations of different baking conditions and technologies on the properties of bread rolls**

Analyzing the effects of the baking conditions on the digestibility of RS containing bread samples, the most significant factor was the starch quality among the following parameters: baking temperature, weight or the added starch type. The alterations of the other parameters (30 °C and 60 °C differences in the values of temperature or 75 g and 150 g differences in the values of weight) did not cause significant changes in the kinetic parameters in the applied test.

The freezing of the pre-baked breads did not influence the digestibility of RS containing bread rolls; however, the resistant starches probably damaged during dough freezing and two-step freezing (freezing after pre-baking and freezing after full-baking). Moreover, the different freezing and baking technologies did not influence the sensory properties of samples. The volumes of samples did not differ from each other except in case of bread rolls prepared from frozen doughs which values were higher compared to the samples without freezing.

#### **4. THESES**

**I.** This was the first study in which the physicochemical properties of resistant starches were analyzed using NIR spectroscopy. It has been proved that the resistant starches differ from native starches according to their structural characteristics. Moreover, this study was the first in which the addition of high amylose RS2 highlighted the sensitivity of two different carbohydrate regions (2080–2130 nm; 2275–2290 nm) for the detection of the increasing amount of amylose. The differences of amylose-amylopectin ratio can be observed in the region of 2275–2290 nm the most sensitively (**1**).

**II.** It has been proved that the digestibility of resistant starches changes differently in case of cooking or microwave treatments (**2, 3**). It was shown that the digestibility parameters: the totally liberated glucose during a three-hour period and the rate constant are applicable for the detection of the increasing amylose concentration in case of mixtures of cooked resistant and native starches (**2**). The microwave irradiation did not influence the digestibility of different types of resistant starches (**3**).

**III.** The microwave irradiated resistant starches were not able to gelatinise; however, it has been shown that the rheological properties of these starches are deteriorated. The application of NIR spectroscopy has been proved to be sensitive enough for the detection of the changes of starches indicated by the microwave irradiations. Additionally, I have shown that the dispersive NIR spectrometer can detect the chemical and physical changes more sensitively than the Fourier transform spectrometer despite the latter one's higher resolution. This study was the first which has investigated the microwave treatments caused effects on the enzymatic, rheological and spectroscopic properties of resistant starches. (**3**).

**IV.** It has been presented that the significant correlations between different enzymatic and rheological methods provide useful information for the prediction of *in vitro* digestibility of untreated and cooked native and resistant starch mixtures based on the RVA results. Moreover, the relationship between the methods highlighted the contribution of RVA parameters to the change of enzymatic digestibility in raw and cooked samples containing native and resistant starches (**2**).

**V.** This study was the first, which applied the Mixolab technology for the detection of RS addition (quality decreasing effect) to different flours. Depending on the medium, the parameters of the gelatinisation, breakdown or retrogradation phases are determining. The *C5* parameter, a characteristic Mixolab parameter that is the maximum torque after cooling, can be applied for the prediction of RS addition to flours independently on the medium (5).

**VI.** The digestibility of the RS enriched bread products did not change significantly due to the weight reductions or temperature decreases; however, the RS type was determining factor (12). The results of the experiments carried out on bread rolls underline the relevance of the technology optimization and the necessity of the investigation of all technologies (e.g. freezing) applied in food industry (12, 14).

## **5. PRACTICAL APPLICATION OF THE RESULTS**

The theses underline that the resistant starches can be characterised using new methods in their analysis such as near infrared spectroscopy and Mixolab device. These techniques can broaden the methods used in analysis of RS and RS enriched products. Additionally, this study indicates that the different types of resistant starches behave similarly in case of dry heat treatment (microwave, a newer investigated effect); however, the more intense moisture heat treatment causes different changes in the properties of RSs. Applying model systems, the relationships between enzymatic and rheological methods are highlighted; additionally, the sensitivity of different spectrophotometers can be compared. The results of the bread products allow concluding that the effects of different technologies have to be taken into account. My results confirm the applicability of resistant starches in starch-based products as well as their nutritional role. The newer applied methods can help the food analysts to understand the properties of resistant starches more widely. Moreover, the knowledge of the effects of newer technologies and adjustments can help product developers and food producers to preserve the beneficial properties of resistant starches even after different technological steps.

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## 6. PUBLICATIONS AND PRESENTATIONS

### Article in international journal with impact factor published in English

1. **Hódsági, M.**, Gergely, S., Gelencsér, T. & Salgó, A. (2010) Investigations of native and resistant starches and their mixtures using near infrared spectroscopy. Food and Bioprocess Technology: An international Journal, DOI 10.1007/s11947-010-0491-5. IF(2010): 3.576.
2. **Hódsági, M.**, Gelencsér, T., Gergely, S. & Salgó, A. (2010) In vitro digestibility of native and resistant starches: correlation to the change of its rheological properties. Food and Bioprocess Technology: An international Journal, DOI 10.1007/s11947-010-0391-8. IF(2010): 3.576.
3. **Hódsági, M.**, Jám bor, Á., Juhász, E., Gelencsér, T., Gergely, S. & Salgó, A. (2011) Effects of microwave heatings of native and resistant starches. Acta Alimentaria. IF(2010): 0.379.
4. Gelencsér, T., Juhász, R., **Hódsági, M.**, Gergely, S. & Salgó, A. (2008) Comparative study of native and resistant starches, Acta Alimentaria, 37(2):255-270. IF(2008): 0.441.

### Article in hungarian journal published in Hungarian

5. **Hódsági, M.**, Gelencsér, T. & Salgó, A. (2010) A Mixolab technika alkalmazási lehetőségei. Élelmiszervizsgálati Közlemények, 56(3):141-152.

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

6. Gelencsér, T., Gál, V., **Hódsági, M.** & Salgó, A. (2008) Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta, Food and Bioprocess Technology: An International Journal, 1:171-179. IF(2009):2.238.

### Oral presentation in English

7. **Hódsági, M.**, Gelencsér, T. & Salgó, A. (2008) The effects of different baking conditions on the in vitro digestibility of resistant starch enriched bread rolls. International Scientific Conference on Cereals, Debrecen, Hungary, 27.-28.10.2008.
8. **Hódsági, M.**, Gelencsér, T., Gergely, S., Hevér, A., Jám bor, A. & Salgó, A. (2010) Some novel basic and applied researches of resistant starches. 9<sup>th</sup> European Young Cereal Scientists & Technologists Workshop, Budapest, Hungary, 25.-27.05.2010.

### Oral presentation in English not used in the dissertation

9. **Hódsági, M.**, Gelencsér, T., Gergely, S. & Salgó, A. (2009) Investigations of the effects of different storage conditions on native and resistant starches. 8<sup>th</sup> European Young Cereal Scientists & Technologists Workshop, Viterbo, Italy, 03.-05.08.2009.

10. Gelencsér, T., Gál, V., **Hódsági, M.**, Smeets, A., Lejeune, M., Westerterp-Plantega, M. & Salgó, A. (2008) Investigations of pasta products prepared with and without resistant starch addition. International Scientific Conference on Cereals, Debrecen, Hungary, 27.-28.10.2008.

Oral presentation in Hungarian

11. **Hódsági, M.**, Gelencsér, T., Gergely, S., Hevér, A., Jámbor, A. & Salgó, A. (2010) Rezisztens keményítők alap és alkalmazott kutatásai. 7<sup>th</sup> Conference of PhD students (BUTE), Faculty of Chemical and Bioengineering, Budapest, Hungary, 04.02.2010.

Proceeding of oral presentation in proceedings of international conference in English

12. **Hódsági, M.**, Gelencsér, T. & Salgó, A. (2008) The effects of different baking conditions on the in vitro digestibility of resistant starch enriched bread rolls. International Scientific Conference on Cereals, Debrecen, Hungary, 27.-28.10.2008. ISBN 978-963-9732-38-4, pp 274-279.

Proceeding of oral presentation in proceedings of international conference in English not used in the dissertation

13. Gelencsér, T., Gál, V., **Hódsági, M.**, Smeets, A., Lejeune, M., Westerterp-Plantega, M. & Salgó, A. (2008) Investigations of pasta products prepared with and without resistant starch addition. International Scientific Conference on Cereals, Debrecen, Hungary, 27.-28.10.2008. ISBN 978-963-9732-38-4, pp 79-83.

Proceeding of poster presentation in proceedings of international conference in English

14. **Hódsági, M.**, Hevér, A., Gelencsér, T. & Salgó, A. (2009) The effects of different baking and freezing technologies on the in vitro digestibility of resistant starch enriched bread products, 5<sup>th</sup> International Congress & 7<sup>th</sup> Croatian Congress of Cereal Technologists "Flour-Bread '09", Opatija, Croatia, 21.-23.10.2009. ISBN 978-953-7005-21-4, pp 210-218.

Abstract in book of abstracts of international conference in English

15. **Hódsági, M.**, Gelencsér, T., Gergely, S., Hevér, A., Jámbor, A. & Salgó, A. (2010) Some novel basic and applied researches of resistant starches. 9<sup>th</sup> European Young Cereal Scientists & Technologists Workshop, Budapest, Hungary, 25.-27.05.2010, p. 41.

Abstract in book of abstracts of international conference in English not used in the dissertation

16. **Hódsági, M.**, Gelencsér, T., Gergely, S. & Salgó, A. (2009) Investigations of the effects of different storage conditions on native and resistant starches. 8<sup>th</sup> European Young Cereal Scientists & Technologists Workshop, Viterbo, Italy, 03.-05.08.2009, p. 17.

Poster presentation in English

17. **Hódsági, M.**, Gelencsér, T. & Salgó, A. (2008) Investigations of resistant starches, Brussel, Belgium, DioGenes project, 12.-14.03.2008.
18. **Hódsági, M.**, Gelencsér, T. & Salgó, A. (2008) Investigations of resistant starches, 5<sup>th</sup> Conference of PhD students (BUTE), Faculty of Chemical and Bioengineering, Budapest, Hungary, 08.02.2008.
19. **Hódsági, M.**, Hevér, A., Gelencsér, T. & Salgó, A. (2009) The effects of different baking and freezing technologies on the in vitro digestibility of resistant starch enriched bread products, 5<sup>th</sup> International Congress & 7<sup>th</sup> Croatian Congress of Cereal Technologists "Flour-Bread '09", Opatija, Croatia, 21.-23.10.2009.

Poster presentation in English not used in the dissertation

20. **Hódsági, M.**, Gelencsér, T., Gergely, S. & Salgó, A. (2009) Investigations of the storage of native and resistant starches, 6<sup>th</sup> Conference of PhD students (BUTE), Faculty of Chemical and Bioengineering, Budapest, Hungary, 04.02.2009.