



# Production of new immobilized $\beta$ -glucosidase and study on its food industrial applicability

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## PhD School/Program

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**The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defense process.**

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## 1. INTRODUCTION

Enzymes in nature have been used for food production (e.g. for the production of cheese, beer, wine and vinegar) for a long time. The purification and application of enzyme preparations became possible at the end of the nineteenth century as a result of the development in fermentation technologies. Currently most enzymes in industrial utilizations are hydrolases with a hydrolyzing role of different biomolecules. One of the biggest groups of hydrolases is the carbohydrate degrading enzymes.

Hydrolase  $\beta$ -glucosidase can be used in the food industry for the hydrolysis of *O*-glucosides (e.g. cellobiose and saccharides with food quality decomposing properties). It can be also used for the reverse hydrolytic synthesis of oligosaccharides and *O*-glycosides, release of aromatic compounds from glycosidic bond and degradation of  $\beta$ -glucan from different cereals. As enzymes (as  $\beta$ -glucosidase as well) are proteins, they can be sensitive to salt concentration, heat and pH of the utilization medium. Therefore they need optimized parameters to retain their stability. Sometimes enzymes in foods can be contaminant when they cannot be removed. Enzyme immobilization can provide many important advantages over the application of native enzymes, namely reusability, continuous operation, controlled product formation, easy separation, use in non-conventional media and simple processing. Enzyme immobilization also could increase the stability of enzymes. Many kinds of immobilization methods for  $\beta$ -glucosidase were published earlier. In these methods mostly expensive or problematic carriers and complicated immobilization technologies were used. Moreover several immobilized  $\beta$ -glucosidase preparations described in the literature could not be used in the food industry. Therefore it is necessary to synthesize a new, cheap  $\beta$ -glucosidase preparation that can be prepared easily and used in the food industry.

The applicability and synthetic activity of  $\beta$ -glucosidase preparations immobilized to different carriers in reverse hydrolytic processes were tested earlier in the Department of Applied Chemistry under the direction of Prof. Dr. Judit Kosáry. Under her supervision Teréz Balogh in her PhD work used  $\beta$ -glucosidase immobilized on a modified polyacrylamide-type bead support (Acrylex C-100) for reverse hydrolysis of environmentally friendly surfactant *O*-alkyl glucosides used in the food industry.  $\beta$ -Glucosidase was immobilized on S-layer followed by entrapping in calcium alginate gel. She used this preparation for flavor enrichment of wines.

## 2. OBJECTIVES

The aim of my work was to produce a completely new immobilized  $\beta$ -glucosidase preparation for different food industrial applications, and to work out a simple and cheap immobilization method. I planned seeded attending to the study on immobilization method (a two-step procedure where after the adsorption of the enzymes to the surface of an insoluble carrier – Amberlite IRA 900 resin - a cross-linking process by a bifunctional reagent as glutaraldehyde is used) since this immobilization method has not been used earlier for  $\beta$ -glucosidase. The connection between the strength of the created bonds and the parameters of the immobilization method has not been studied earlier either. In the interest of it a new examination method had to be developed. In order to achieve my above mentioned aims I formulated the main objectives:

1. Sort out the most convenient  $\beta$ -glucosidase activity assay.
2. Select carrier for the immobilization method that is cheap, easily available, furthermore has convenient physical and chemical properties. I planned to develop an economical immobilization method that needs simple technology.
3. Develop a special method for the study of the enzyme immobilization and to characterize the strength and the properties of forces between the enzyme and the carrier that can make possible the determination of the convenient parameters of the immobilization method.
4. Based on theoretical considerations select the parameters that can mostly influence the immobilization method and determine their convenient rate by experimental examinations.
5. Examination of the developed immobilization technique and the characterization of the immobilized enzyme.
6. Test the applicability of the developed enzyme preparation in some food industrial utilizations and based on scientific and experimental consideration select an application and carry out detailed examinations on it. The planned food industrial utilizations: cellobiose degradation, applicability in wine production circumstances, reverse hydrolysis to produce *O*-alkyl glucosides and barley  $\beta$ -glucan oligosaccharides degradation.

## 3. MATERIALS AND METHODS

The experimental part of the PhD work was carried out at the Department of Applied Chemistry at the Corvinus University of Budapest. Experiments and research work related to the study on applicability of native and immobilized  $\beta$ -glucosidase on barley  $\beta$ -glucan were carried out in Norway at Nofima Mat (The Norwegian Institute of Food Research). For the study on the immobilization

method and the applicability of the immobilized enzyme in food industrial utilizations  $\beta$ -glucosidase (Fluka) isolated from almond (EC 3.2.1.21) was used. The barley  $\beta$ -glucan hydrolysate was produced by glucanase enzyme (Megazyme) isolated from *Bacillus subtilis* (EC 3.2.1.73).

### **3.1 Enzyme immobilization method**

Before the immobilization method the Amberlite *IRA-900* (anion-exchange macroreticular resin) hydroxide form (Rohm & Haas) was prepared by washing the chloride with sodium hydroxide. A two-step immobilization method was applied. At first the enzyme was adsorbed to the modified carrier followed by a cross-linking with glutaraldehyde to stabilize the enzyme protein structure on the surface. The parameters of the immobilization were optimized separately for both steps.

### **3.2 $\beta$ -Glucosidase activity assay**

The activity of native or immobilized  $\beta$ -glucosidase was determined by using *p*-nitrophenyl- $\beta$ -glucopyranoside (*p*NPG) as substrate and the activity was calculated by the concentration of released *p*-nitrophenol (was measured at 400 nm). In one experiment cellobiose was used as substrate and the activity was calculated on the basis of the released glucose that was measured by enzymatic kit.

### **3.3 Kinetic desorption method**

In this technique the measurement of enzyme activity was repeated several times in which defined short terms for activity measurements were used. After the first activity measurement the preparation was separated (filtrated) from the reaction mixture, washed and its activity was re-measured in a fresh reaction mixture. The activity of the filtrate of previous reaction mixture was also measured. This cycle was then repeated. Based on the change in the activities of immobilized enzyme between the cycles it was possible to characterize the strength of bonds between the enzyme and the carrier.

### **3.4 Study on applicability in the hydrolysis of cellobiose**

A model solution was created that contained the cellobiose as substrate and the efficiency of the process was calculated on the basis of the amount of liberated glucose. Since in the industry the applied microorganisms have different pH optimum for the degradation of cellobiose therefore I also carried

out the experiments with the enzyme preparations (native and immobilized) by using different pH values.

### **3.5 Study on applicability in wine production circumstances**

The effect of pH and ethanol content of the solution to the catalytic activity was examined by using *p*NPG substrate in wine sample and in different model solutions. On the basis of theoretical and experimental considerations the application stability of the immobilized enzyme was studied by using cellobiose as substrate.

### **3.6 Study on applicability in the reverse hydrolytic synthesis of *O*-glycosides**

The reaction mixtures contained different alcohols (n-hexanol, 2-butoxy-ethanol, butanol) and 1,2-diacetoxy-ethane and in one case only butanol was the component. All reaction mixtures contained 10 % water and the glucose concentration was 10 mM. The efficiency of the process was calculated on the basis of the decreasing amount of glucose in the system furthermore the retained activities of the enzyme preparations (native and immobilized) were also followed during the three days reaction.

### **3.7 Study on applicability in the degradation of barley $\beta$ -glucan**

Determination of  $\beta$ -glucan di- and oligosaccharides was carried out by using High-Performance Anion-exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD) and in contrast to the earlier literature practice for the quantitative determination internal standard and barley  $\beta$ -glucan di- and oligosaccharide standards (newly available in commerce) were utilized.

This approach allowed me to examine the operation and compare the kinetic characteristics of the enzyme preparations (native and immobilized) on each exclusive oligosaccharide substrate and on glucanase degraded barley  $\beta$ -glucan. The kinetic parameters of the enzyme preparations were also determined in these systems on the basis of the released end products and the substrate as well.

### **3.8 Chemicals and Statistical Analysis**

All the experiments were repeated at least two times (because of the high number of experiment) and results were reproducible and the differences were between  $\pm 5$  %.

## 4. RESULTS AND DISCUSSION

### 4.1 Selecting the $\beta$ -glucosidase activity assay

As first step of my work I selected the most convenient method to measure enzyme activity. I examined two methods where cellobiose and *p*-nitrophenyl- $\beta$ -D-glucopyranozide (pNPG) are used as substrates. Considering cost performance and time demand the pNPG method was more appropriate for my further research. By using pNPG substrate the applied enzyme showed maximum activity ( $10.54 \mu\text{mol min}^{-1} \text{ enzyme mg}^{-1}$ ) at pH 5.50.

### 4.2 Examination of the immobilization method

*Amberlite IRA 900 Cl* anion-exchange resin as a carrier has never been used before for the immobilization of  $\beta$ -glucosidase (earlier it was applied for the immobilization of carbohydrate degrading enzyme mixtures because of its good mechanical properties, cheapness and easy availability). A two-step immobilization method was applied. At first the enzyme was adsorbed to the carrier where Van der Waals and ionic bonds and hydrogen bridges could be formed between the enzyme and the carrier. Since these bonds are not strong enough (especially in the presence of high ion and substrate concentration) a cross-linking between the enzyme molecules with glutaraldehyde to stabilize the enzyme protein structure on the surface was applied.

This technique applied two different kinds of chemical interactions for immobilization. Therefore the parameters of the immobilization were optimized separately for the two steps since each could require different parameters. For this optimization I developed a quick and efficient method that permitted the studying of the strength and the properties of the forces between the enzymes and support *via* the examinations of the kinetic of desorption and/or inactivation. By this technique called kinetic desorption method the effect of reaction parameters on immobilization could also be studied and it was possible to determine the prosperous values of these parameters.

#### 4.2.1 Examination of the adsorption method

At first the examination of parameters that can mostly influence the adsorption was carried out by examination of pH, salt concentration, temperature and the presence of substrate. By applying the kinetic desorption method in all cases a decrease in the immobilized activity was found between the cycles because of desorption of the enzyme molecules. For this reason the highest immobilized activity was observable every time in the first cycle after the immobilization and this activity was stabilized in

the cross-linking process by glutaraldehyde. Contrary to the expectation the best results were detected for the highest buffer concentration (0.1 M sodium acetate buffer) in the first cycle. It was found that the higher buffer concentration resulted in higher pH value in the reaction mixture at the end of the immobilization process which fact could be attributed to a supposed partial hydroxide-acetate anion change. As  $\beta$ -glucosidase has an isoelectric point at pH 5.50 therefore the high pH value (8.2) at the end of the immobilization process could generate higher negative charge for the enzyme. It is supposed this higher negative charge of the enzyme caused the better connection to the anion exchange resin in contempt to high buffer concentration. It was found that the presence of substrate (cellobiose as a natural substrate of  $\beta$ -glucosidase) and the temperature (between 10 and 30 °C) had no considerable effect on the adsorption process by using the chosen parameters (0.1 M sodium acetate buffer pH 5.50). support-enzyme ratio 10:1 and 24 h reaction time). I examined in the case of a given amount of carrier how the applied amount of enzyme could influence the immobilized activity. The maximum yield of immobilized enzyme activity was 22.5 % while by using the general ratio of carrier:enzyme (10:1) it was given to 17.7 %.

#### ***4.2.2 Examination of the glutaraldehyde treatment***

In the second step the examination of parameters that can mostly influence the cross-linking by glutaraldehyde was carried out by the examination of time of treatment, pH, glutaraldehyde concentration and the presence of substrate. By using the kinetic desorption method at first an increase in the immobilized activity was found. This enhance is attributed to an advantageous change in the structure of the enzyme layer during operations after the cross-linking reaction because of the presence of substrate. It was observed that the presence of substrate and the pH had no considerable effect on the cross-linking process. For the glutaraldehyde treatment the convenient parameters were chosen as follows: concentration (0.1 M) of the buffer (sodium acetate pH 5.50) time of treatment (1 min) and the concentration (0.25 m/v %) of glutaraldehyde. During my studies it was found that too high concentration or time of treatment resulted in considerable decrease in the immobilized activity while in the case of too low glutaraldehyde concentration a minimal effect of cross-linking reaction was found (similar desorption was observable than for adsorption). I examined in the case of a given amount of carrier how the applied amount of enzyme could influence the immobilized activity for this combined technique. The maximum yield of immobilized enzyme activity was 21.4 % while by using the general ratio of carrier:enzyme (10:1) it was given to 5.4 %. During the examination of the optimum pH for the catalytic activity of the immobilized enzyme, under pH 5.0 the results of catalytic activity measurement were not reproducible that suggested stability problems as a result of the pH



value. This fact could be attributed to the changed electrostatic circumstances because of the enzyme could act as a polycation under its isoelectric point on the surface of the anion exchange resin. Accordingly increasing the stability of interactions was necessary to obtain a stable immobilized preparation. By increasing the time of glutaraldehyde treatment to 30 min I could create immobilized enzyme with enlarged stability that was stable in the medium even at pH 4.00.

#### **4.3 Characterization of the immobilized enzyme with enlarged stability**

Kinetic parameters and the pH and temperature optimum of catalytic activity of immobilized enzyme were determined during characterization. The  $V_{\max}$  value for the native enzyme was  $15.61 \mu\text{mol min}^{-1} \text{mg enzyme}^{-1}$  and the  $K_M$  value was 4.04 mM, while for the immobilized preparation  $V_{\max}$  was  $75.64 \mu\text{mol min}^{-1} \text{g dry resin}^{-1}$  with a low yield of immobilized enzyme activity (2.1 %) because of the high amount of utilized enzyme for the immobilization (carrier:enzyme ratio 10:3) and the  $K_M$  was 5.03 mM. The results suggested that the immobilization process mostly affected the catalytic properties of the enzyme but not due to the modification of enzyme structure by the immobilization method but the enzyme molecules were immobilized in inconvenient position.

The immobilization process caused change in the pH optimum from pH 5.50 to 4.50. In the polycationic microenvironment resulted by the surface of the carrier (Amberlite IRA 900) the  $\text{H}^+$  ion concentration could be lower than in the external macro-environment that could result in the change of the optimal pH value at the acidic range.

The immobilized enzyme preparation of increased stability had an optimum at higher temperature (60 °C) for the catalytic activity than the native enzyme did (50 °C). On the basis of the literature it is supposed that the macroporous structure of the carrier, the created ionic and covalent bonds and diffusive effects could protect the immobilized enzymes against the heat inactivation.

#### **4.4 Studies on food industrial applicability of the immobilized enzyme with enlarged stability**

##### ***4.4.1 Study on applicability in the hydrolysis of cellobiose***

One of the most important utilizations of  $\beta$ -glucosidase is in glucose production from cellulose by using cellulase and the produced glucose can be a departure point for different industrial processes. In the created model solutions (with different pH) the yield of the process was considered 100 % when the presented cellobiose was totally hydrolyzed. I found that the immobilized enzyme was more efficient at pH 5.50 than the native enzyme since at 70<sup>th</sup> hours (the end of the process) it had higher yield (74.2 %) than the native enzyme did (59.9 %) despite the fact that the immobilized preparation

had pH optimum at pH 4.50. As there was no observable difference in the retained activity of the native and immobilized enzyme preparations at the end of the process (69.9 and 73.6 %) I supposed that the immobilized enzyme was more effective on cellobiose than on the artificial pNPG substrate. There was a major difference between the yields of the native and immobilized enzymes (44.4 and 71.5 %) at the end of the reaction. The explanation of this (over the more efficient applicability on cellobiose) that the immobilized enzyme has a lower pH optimum (pH 4.50) and it had higher retained activity (67.5 %) at the end of the process than the native enzyme did (48.5 %). In summary the immobilized enzyme could retain high activity under pH 5.50 (optimal for native enzyme) and because of the immobilization process it could withstand more efficiently the inactivation effect of the acidic pH.

#### ***4.4.2 Study on applicability in wine production circumstances***

Since in wines the most aromatic compounds contain alcoholic hydroxyl group in  $\beta$ -glycosidic bonds therefore the hydrolytic activity of  $\beta$ -glucosidase has a very important role in the release of the aromatic compounds. As a first step I examined the effect of pH and ethanol content (characteristic for wines) on the catalytic activity of native and immobilized  $\beta$ -glucosidase. For the native enzyme both the acidic pH (4.00) and the ethanol content [10 % (V/V)] resulted in notable decrease in the catalytic activity (43 and 55 % retained activity). I found that acidic pH and ethanol content had an additive inactivation effect. In the wine sample further notable decrease was observable in the activity (5 % retained activity) compared to the model solution [pH 4.00 and 10 % (V/V)]. For the immobilized enzyme both the acidic pH and ethanol content had less inactivation effect (82 and 75 % retained activity) than on the native enzyme. Furthermore the acidic pH had less effect on activity of the immobilized enzyme because of the changed pH optimum to the acidic range. The immobilized enzyme could also retain more activity in the wine sample (26 % retained activity) than the native enzyme did. In the second step I examined the operational stability of the immobilized enzyme and I found that in the model solution the enzyme preparation could retain 66 % (after 8 hours) of the measured activity after the first hour while in the case of wine sample this rate was 40 %. On the basis of the results the application of the immobilized enzyme in wine production circumstances can be more advantageous than the utilization of native enzyme.

#### ***4.4.3 Study on applicability in the reverse hydrolytic synthesis of O-glycosides***

The efficiency of the reverse hydrolytic process was monitored on the basis of the available glucose in the system. The yield is 100 % if there is no detectable glucose in the system because they are in *o*-glycosidic bonds. I found that the immediate inactivation of the immobilized enzyme was

smaller scale in every reaction medium than the native enzyme did. The immobilization method could increase the retained activity of the immobilized enzyme by 28-42 %. Furthermore it was also observable that the different reaction mediums had less inactivation effect therefore the immobilized enzyme could retain higher activity at the end of the reaction (after three days). By using the same enzyme activity (in the case of native and immobilized enzymes) at the end of the reaction the immobilized enzyme showed better yield (58-77 %) than the native enzyme did (31-49 %) depending on the reaction medium. The higher yield for the immobilized enzyme could be in line with the increased stability, higher retained activity and the hydrophobe properties of the carrier that could result in more efficient operation in the applied heterogene, apolaric reaction solution. On the basis of the presented results the application of this immobilized preparation was more advantageous than the native enzyme.

#### ***4.4.4 Study on applicability in the degradation of barley $\beta$ -glucan***

In some cases the food industrial technologies require the degradation of  $\beta$ -glucan that can be carried out by enzymatic hydrolysis. Furthermore the complete degradation of  $\beta$ -glucan makes way to food industrial process where the purpose is ethanol production from glucose. Different barley species can be considered as the main source of  $\beta$ -glucans. Glucanase hydrolyses the 1 $\rightarrow$ 4 linkage of the 3-*O*-substituted glucose units of water soluble barley (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)- $\beta$ -D-glucan ( $\beta$ -glucan). The major hydrolysis products (ca. 90%) are 3-*O*- $\beta$ -cellobiosyl-D-glucose (G4G3G) and 3-*O*- $\beta$ -cellotriosyl-D-glucose (G4G4G3G).  $\beta$ -glucosidase can only liberate glucose molecules from the non-reducing ends of the  $\beta$ -glucan oligosaccharides. This action causes monomeric glucose and G4G3G from G4G4G3G, glucose and laminaribiose (G3G) from G4G3G and two glucose monomers from G3G. In general the kinetic parameters of an exohydrolase (as  $\beta$ -glucosidase) on substrates built up from glucose molecules are determined on the basis of liberated reducing ends or the amount of liberated glucose. In the case of substrates (built up from glucose) larger than disaccharides and/or containing different linkages the determination of kinetic parameters on the basis of the liberated glucose may lead to error in calculations since it is impossible to follow the amount of substrates. General methods cannot be used in systems where di- and oligosaccharides are presented at the same time as substrates. Therefore I developed a chromatographic method using HPAEC-PAD to quantify accurately (by melibiose as internal standard and using commercially newly available  $\beta$ -glucan di- and oligosaccharides standards) the major oligosaccharides derived from lichenase degradation of barley  $\beta$ -glucan. This method was used to follow  $\beta$ -glucosidase degradation and product formation as progress curves. This approach allowed me to be the first to compare the kinetic characteristics of  $\beta$ -glucosidase (native and immobilized) on

each exclusive oligosaccharide substrate and their mixtures. As expected the kinetic parameters that were determined for exclusive oligosaccharide substrates were different depending on whether the calculation was based on end product formation or substrate depletion. These differences were mostly affected by the applied substrates. In the case G3G substrate where only one  $\beta$ -(1 $\rightarrow$ 3) bond was hydrolyzed there was no notable difference in kinetic parameters. When G4G3G was the substrate at the start of the reaction the liberated and accumulated amount of G3G could affect (decrease) the amount of liberated end product (glucose) hence the kinetic parameters also showed notable differences based on different calculations. Both  $K_{cat}'$  and  $K_m'$  showed approximately half the rate when they were calculated on the basis of the liberated end product. When G4G4G3G was the substrate the same effect was observable but since the G4G3G and G3G were liberated and presented in a low amount the differences in kinetic parameters (based on different calculations) were smaller. The  $K_m'$  rates were approximately the same while in  $K_{cat}'$  a 20 % decrease was observable compared to the rate that was calculated on the basis of the amount of substrate. By using the developed analytical method I could be the first to characterize the operation of  $\beta$ -glucosidase on barley  $\beta$ -glucan substrates. The experiments were also carried out by the immobilized enzyme and I found that the immobilization method could strongly affect the catalytic activity of the enzyme (based on the above mentioned reasons) while the  $K_m'$  values and the general operation showed the same characteristic.

## 5. NEW SCIENTIFIC ACHIEVEMENTS

**1. Development of the kinetic desorption method.** For the examination of the applied two-step immobilization method I worked out a special method. With the modification of operational stability method (published in the literature) I created another cyclic method called kinetic desorption method [RADVA et al., 2011]. This method permitted the examination of activity of the immobilized enzyme preparation and on the basis of the “desorption of the immobilized enzyme activity” the stability of the immobilized enzyme could be characterized at the same time. This technique was very efficient for the examination of the applied immobilization method since the two different chemical bonds for the two different steps of the immobilization could be examined separately. In summary this new method made possible to follow the effect of the different steps and parameters of the immobilization method on the activity and stability of the immobilized enzyme.

**2. Production and characterization of the new immobilized  $\beta$ -glucosidase enzyme preparation.** I was the first to immobilize  $\beta$ -glucosidase enzyme preparation in a two step method by adsorption on a macroporous carrier (*Amberlite IRA 900 Cl*) followed by a cross-linking of the enzymes with

glutaraldehyde that resulted in a more stable enzyme structure on the surface of the carrier. This requires simple technology and the carrier is easily available and cheap. For the determination of the optimal parameters of the immobilization method I applied the developed kinetic desorption method. I also carried out the characterization of the produced immobilized enzyme where the kinetic parameters, the pH and temperature optimum of the catalytic activity were determined [RADVA & KOSÁRY, 2011].

**3. Studies on food industrial applicability of the new immobilized enzyme.** I studied the applicability of the new immobilized enzyme in four different food industrial utilizations and I carried out detailed examination on a selected utilization. In the case of wine production circumstances, cellobiose degradation and reverse hydrolytic processes I found that the immobilized enzyme could retain its activity more efficiently therefore it was considered as a more stable enzyme preparation than the native enzyme. Due to the higher retained activity and the changed pH optimum the application of the immobilized enzyme was more efficient in the mentioned food industrial processes.

**4. Development of new chromatography method (HPAEC-PAD) for the measurement of barley  $\beta$ -glucan di- and oligosaccharides.** For the examination of barley  $\beta$ -glucan degradation by  $\beta$ -glucosidase I developed a new measurement method that permitted the qualitative and quantitative determination of barley  $\beta$ -glucan di- and oligosaccharides. This technique made possible to follow  $\beta$ -glucosidase degradation and product formation as progress curves. Differently from literature practice I used melibiose as internal standard and for the quantitative determination I applied newly available (in commerce)  $\beta$ -glucan di- and oligosaccharide standards instead of the adequate maltooligosaccharides [RADVA et al., 2012].

**5. Characterization of barley  $\beta$ -glucan di- and oligosaccharides degradation by native and the immobilized  $\beta$ -glucosidase.** By the developed measurement method using HPAEC-PAD I could be the first to carry out examinations on the action of  $\beta$ -glucosidase (native and immobilized) on barley  $\beta$ -glucan substrates. During this examination I studied the action of  $\beta$ -glucosidase on the pure barley  $\beta$ -glucan di- and oligosaccharide and on glucanase degraded barley  $\beta$ -glucan substrates. I also determined the kinetic parameters of the enzymes preparations on these substrates. On the basis of the results I came to the conclusion that following the progress curves of these kinds of substrates was necessary for calculation of kinetic parameters since calculations based specific release of monosaccharide or reducing sugar products in general may lead to error [RADVA et al., 2012].

## 6. RELATED PUBLICATIONS

### **Publications with Impact Factor**

Radva, D., Knutsen, S.H., Kosáry, J., Ballance, S. (2012) : Application of high-performance anion-exchange chromatography with pulsed amperometric detection to compare the kinetic characteristics of  $\beta$ -glucosidase on oligosaccharides derived from lichenase digested barley  $\beta$ -glucan. Carbohydrate Research 358. 56-60. IF<sub>2012</sub>: 2.044

Radva, D., Kosáry, J. (2011) : Production of new immobilized  $\beta$ -glucosidase preparation for food industrial purposes by using kinetic desorption method. Acta Alimentaria 40. 176-187. IF<sub>2011</sub>: 0.444

Radva, D., Spanyol, J., Kosáry, J. (2011) : Testing of the effect of parameters on the enzyme immobilization by adsorption and cross-linking processes with kinetic desorption method. Food Technol. and Biotechnol. 49. 257-262. IF<sub>2011</sub>: 1.195

### **Hungarian conference (summary)**

Radva, D., Kosáry, J.: Élelmiszeripari felhasználásra alkalmas, új rögzített  $\beta$ -glükózidáz enzimmészítmény előállítás. Lippay–Ormos-Vas (LOV) Tudományos Ülésszak, Budapest, 2009. (abstract)

Radva, D., Spanyol, J., Kosáry, J.: Új, Amberlite IRA 900 gyantára rögzített  $\beta$ -glükózidáz enzimmészítmény élelmiszeripari alkalmazhatóságának vizsgálata. Lippay–Ormos-Vas (LOV) Tudományos Ülésszak, Budapest, 2009. (poster)

### **International conference (summary)**

Radva, D., Knutsen, S.H., Kosáry, J., Balance, S.: Application of HPAEC-PAD for the examination of the action of native and immobilized  $\beta$ -glucosidase preparations on barley  $\beta$ -glucan substrate. Aegean Analytical Chemistry Days (AACD 2010), Lesvos Greece, 2010. (abstract)