THE STUDY OF THE APPLICABILITY OF SOME HYDROLASES: α- AND β-GLUCOSIDASES AND PECTINMETHYLESTERASE IN FOOD-INDUSTRIAL TYPE OF PROCESSES

Theses of the doctoral dissertation of

TERÉZ BALOGH

CORVINUS UNIVERSITY OF BUDAPEST
Department of Applied Chemistry

Budapest, 2004

Details of doctoral school

Name:	**doctoral School of Food Science**
Field:	**Food Science**
Head:	**Dr. András Fekete** university professor, DSc Corvinus University of Budapest Department of Physics and Automation
Supervisor:	**Prof. Dr. Judit Kosáry** Corvinus University of Budapest Department of Applied Chemistry

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

.....	**.....**
Head of PhD School	**Supervisor**

1. INTRODUCTION

1.1 How enzymes were used in food industry

Natural enzymes have been used for making food (e.g. for the production of cheese, leaven, beer, wine and vinegar) for a long time. Enzymes were also made use of in other industrial sectors (e.g. leather and flax industry, manufacturing of indigo). However, the preparation and utilization of practically pure enzyme preparations became possible only at the end of the nineteenth century as a result of the development in fermentation technologies. The use of enzymes in different technologies based on scientific results was started by using takadiastase in the USA (1984). In the last four decades the development of enzymology was in connection with that of biotechnology.

Nowadays most enzymes of industrial consumption are hydrolases (third class of enzymes) with a hydrolyzing function of different biomolecules. In the food industry the most frequently used enzymes are proteases, amylases, lipases (cheese industry), lactases (milk industry), pectinmethylesterases (fruit and vegetable preparations), pectinases and transglutaminases.

Enzymes play an important role in the modern food industry and they are used in order to solve various combined problems. No wonder that the enzyme industry, connected with enzyme research, developed parallel with the food industry. However, the use of enzymes in manufacturing processes has several drawbacks. As enzymes are proteins, they can be sensible to heat, salt concentration and pH of the reaction mixtures. Therefore they need optimized parameters to retain their stability. Sometimes enzymes in foods can be pollutants when they cannot be removed. The immobilization of enzymes increases their stability, makes their removal easier and renders their recycling possible; therefore the costs can be reduced.

There are other problems in connection with enzymes in food-making processes. As the raw materials in food industry were originally living organisms with more or less active endogenous enzymes, their deactivation can be necessary. Because of the activity of endogenous enzymes in fruits and vegetables the quality can easily deteriorate during their storage and transport, causing serious problems in food-making processes. In traditional food-industrial processes the only solution for these problems was heat treatment, not only for the deactivation of endogenous, thermosensitive enzymes but also for the sterilization of foods.

In recent years the quality conditions of foods have changed significantly, because of the assertion of consumers' claims. There are important points of view in the modern qualification of foods: to preserve the original properties of the raw materials using only few and gentle technological operations in food-making processes. Foods produced by traditional food-

industrial processes did not meet these requirements and new and gentler procedures and technological processes had to be worked out in order to satisfy the demand.

1.2 Objectives

This dissertation covers studies of the applicability of some hydrolases: α- and β-glucosidases and pectinmethylesterase in food-industrial type of processes. A characteristic food-industrial process was designed for both glucosidases and pectinmethylesterase. On the basis of the earlier results in the Department of Applied Chemistry the PhD candidate Teréz Balogh worked on five main objectives:

- *O*-Alkyl-glucosides are non-ionic, non-toxic surfactants, those are suitable for biological degradation. Some of them are used as detergents in food industry. The synthesis of *O*-alkyl-glucosides by reverse hydrolytic processes from glucose and hydroxyl compounds (as glucosyl acceptors) in organic solvents (as diluting agents) was planned even in preparative scale. Those kinds of reactions expanding the group of the hydroxyl compound used have not been carried out in this way earlier.
- The planned model system for the application of glucosidases in food industry was to use them for flavour enrichment of wines.
- The formation of a new immobilized β-glucosidase preparation was planned using the surface layer as carrier. Surface layer has not been used for enzyme immobilization earlier.
- The planned model system for the application of pectinmethylesterase in food industry was to attempt the quality amelioration of vegetables, especially carrot preparations by the deactivation of endogenous pectinmethylesterase on the basis of the results of deactivation kinetics studies on native (isolated, purified and lyophilized) carrot pectinmethylesterase.
- The activity of endogenous enzymes in raw materials of plant origin in food industry can be informative for their storage, transport and use. Therefore the study of the change in the activity of endogenous β-glucosidase and pectinmethylesterase in different raw materials of plant origin at the conditions characteristic for the effect of some processes in food industry was planned.

2. MATERIALS AND METHODS

2.1 Assay of the activity and study of the stability of α- and β-glucosidases

The activity of native (commercially available, lyophilized) α- and β-glucosidases and different immobilized α- or β-glucosidase preparations were determined by an indirect way *via* measuring

the change in the glucose concentration of the reaction mixtures. For the determination of glucose concentrations the glucose-6-phosphate dehydrogenase-NADP enzymic system with a special individual modification was used. The activity unit of α- and β-glucosidase activities was defined as the amount of enzyme (mg protein for native or g support for immobilized enzymes) required to release 1 μmol glucose per minute from substrate sucrose or cellobiose in sodium acetate buffer (0.1 M, pH 5.1). The stability of α- and β-glucosidases in the organic solvents of the planned reverse hydrolytic processes was characterised by the kinetic studies of immediate and incubation deactivations.

2.2 Assay of the activity and study of the stability of pectinmethylesterase

The activity of native (isolated, purified and lyophilized) pectinmethylesterase and the pectinmethylesterase content of different carrot preparations were evaluated by titrating the free carboxyl groups released from a pectin solution with sodium hydroxide. The activity unit of pectinmethylesterase activity was defined as the amount of enzyme required releasing 1 μmol carboxyl group (expressed in μmol sodium hydroxide) in per minute from 0.5% apple pectin solution (30 ml) at pH 7.0 and 35 °C. The stability of native pectinmethylesterase and the pectinmethylesterase content of different carrot preparations was characterised by the kinetic studies of deactivation results during heat and high-pressure treatment practically equal to the applications planned for different carrot preparations.

2.3 Study of the activity of endogenous α- and β-glucosidases and pectinmethylesterase activity in food-industrial raw materials of plant origin

Different commercial food raw materials produced in Hungary were studied: carrot (*Daucus carota*), broccoli (*Brassica oleracea*), vitis (*Vitis vinifera*), cauliflower (*Brassica cretica*), sweet almond (*Prunus amygdalus*). For activity assays of different enzymes before and after treatment in different extractions were made. For α- or β-glucosidase assays extractions (100 mg ml^{-1}) generally used for lipoxigenases were made with 0.05 M TRIS acetate buffer pH 8.2 containing sucrose (0.38 M) and calcium chloride (0.02 M). For pectinmethylesterase assays the extraction method (1 g ml^{-1}) known in the literature with some special individual modifications was used with 0.5 M TRIS acetate buffer pH 8.5 containing Triton-X-100, glycerol, sodium chloride, polyvinylpyrrolidone K 25 and dithiothreitol.

2.4 Immobilization of α- and β-glucosidases

The α- and β-glucosidases were immobilized on a modified polyacrylamide-type bead support (Acrylex C-100) possessing carboxylic groups by an optimized carbodiimide method followed by lyophilization. β-Glucosidase was immobilized on surface layer by a special co-cross-linking with glutaraldehyde followed by entrapping in calcium alginate gel.

2.5 Modelling of the applicability of β-glucosidase and pectinmethylesterase in food-industrial type of processes

The immobilized β-glucosidase preparations were used for flavour enrichment of wines. Different Muscat wines were shaken with both β-glucosidase immobilized on Acrylex C-100 support and β-glucosidase immobilized to surface layer then entrapped in calcium alginate gel. The samples were extracted by a mixture of diethyl ether and pentane, the flavour enrichment of wines was tested by gas chromatography.

The use of pectinmethylesterase in food industry was modelled by the quality amelioration studies of different carrot preparations (a mixture for modelling carrot juice made in industrial conditions and carrot slices) *via* deactivation of endogenous pectinmethylesterase in these preparations. The parameters of heat and high pressure deactivation processes were determined on the deactivation results of native pectinmethylesterase.

2.6 Synthesis of surfactant *O*-alkyl-glucosides by reverse hydrolysis

Surfactant *O*-alkyl-glucosides were synthesized with reverse hydrolytic processes catalyzed by α- or β-glucosidases immobilized on Acrylex C-100 support. Different alcohols were used as glucosyl acceptors and for solvent function different kinds of organic solvents were tested. The conversions were estimated in an indirect way by measuring the glucose content of the reaction mixtures. The yields of the synthesis of *O*-alkyl-glucosides were measured by high performance thin-layer chromatography.

During the preparative-scale synthesis of *O*-substituted glucopyranosides by my novel reaction system replacing alcohols as solvents with 1,2-diacetoxyethane an evaporation method and in the case of triacetin solvent an exaction method were used. The products were isolated by thick-layer chromatography and their structure and optical purity were controlled by ^{1}H-NMR spectra.

3. RESULTS AND DISCUSSION

For the modelling of food-industrial applications the kinetic properties and stability relations (immediate deactivation and deactivation kinetics) of native α- and β-glucosidases and pectinmethylesterase, then immobilized α- and β-glucosidase preparations were studied at the conditions practically equal to the applications planned.

3.1 Study of α- and β-glucosidases and pectinmethylesterase

3.1.1. Assay of *O*-glucoside content of reaction mixtures

In connection with α- and β-glucosidases the *O*-glucoside content of reaction mixtures was determined by an indirect way *via* measuring the decrease in the glucose concentration of the reaction mixtures and by a direct method *via* high performance thin-layer chromatography (HPTLC) method. For the measuring of glucose concentrations the glucose-6-phosphate dehydrogenase-NADP enzymic system widely known in the literature was used. To replace expensive commercial diagnostic kits a special individual modification was used for cost-reduction. The principle of the method was retained but some chemical materials and the concentration of reagents were altered.

3.1.2. Study of the stability of α- and β-glucosidases

In aqueous solutions enzymatic hydrolytic reactions catalyzed by hydrolases are practically irreversible, but hydrolysis can be converted to synthesis by reducing the water content of the medium *via* using organic solvents – this is *reverse hydrolysis*. Therefore the stability of α- and β-glucosidases was studied in the organic solvents of the planned reverse hydrolytic processes. The stability of α- and β-glucosidases was characterised in two different ways. In the kinetic studies of *immediate deactivation* the enzyme activities in different reaction mixtures were compared to the original enzyme activity measured in aqueous mixture (it was taken as hundred percent). The effect of reverse hydrolytic reaction mixtures on enzyme stability was characterized by *incubation deactivation.* In the study of deactivation kinetics the loss in enzyme activity during reverse hydrolysis was compared to the enzyme activity at the start of the reaction (immediate deactivation value was taken as hundred percent). Immediate deactivation and deactivation kinetics of native and immobilized α- and β-glucosidases were tested in reaction mixtures containing different water miscible and immiscible organic solvents of water content 5-10-15%, hexanol (10%) as glucose acceptor and glucose (10 mM) as stabilizing agent. Generally the organic solvents tested decreased the stability of α- and β-glucosidases, moreover,

most of them caused an immediate and complete loss in enzyme activity. However, I found stability results similar to data in pure alcohols in the case of both native β-glucosidase and immobilized form on Acrylex C-100 in 1,2-diacetoxyethane or triacetin.

3.1.3. Study of native pectinmethylesterase

In a heat and high-pressure treatment of native pectinmethylesterase the inactivation conditions were practically equal to the applications planned for different carrot preparations. The heat and high-pressure deactivation kinetics of native pectinmethylesterase were characterized by kinetic data analysis and found that native pectinmethylesterase followed a first-order kinetic model in both cases. I found that data of temperature and the pH of the solutions influenced on the decimal reduction time (D) data of the enzyme, as well. The heat and high-pressure deactivation kinetics of native pectinmethylesterase were really fast in acidic solutions (pH 4.5).

3.2. Immobilization of glucosidases

3.2.1. Immobilization of α- and β-glucosidases on Acrylex C-100 support

The parameters of the immobilization of both α- and β-glucosidases on Acrylex C-100 support by carbodiimide method described earlier were optimized. The activity of immobilized β-glucosidase was enhanced from 73 μmol min^{-1} xerogel g^{-1} to 153 μmol min^{-1} xerogel g^{-1} and the yield was altered from 13 % to 27 %. The activity of immobilized α-glucosidase was enhanced from 1 μmol min^{-1} xerogel g^{-1} to 3 μmol min^{-1} xerogel g^{-1} and the yield was altered from 3 % to 4 %. It was found that the activity and yield of the immobilization of α-glucosidase on Acrylex C-100 support were too low for a preparative synthetic method.

3.2.2. Immobilization of β-glucosidases on surface layer (S-layer)

Surface layer isolated from *Bacillus stearothermophilus* PV72 has not been used for enzyme immobilization earlier. It was known that before immobilization on other kind of proteins by carbodiimide method a covalent cross-linking by glutaraldehyde was generally used because of the soft structure of the recrystallized S-layer protein. This cross-linking method was connected with enzyme immobilization: the mixture of S-layer and enzyme was treated together. In this way enzyme was immobilized on surface layer by co-cross-linking with glutaraldehyde. This new method was tested for several enzymes but the yield was higher than 10 % (12.5 %) only in the case of β-glucosidase. The activity of the new β-glucosidase preparation was 82 μmol min^{-1} dry S-layer g^{-1}. The organic solvents tested caused an immediate and complete loss in enzyme

activity of β-glucosidase immobilized on surface layer therefore it was unusable in reverse hydrolytic processes. Because of the protein structure of surface layer support this β-glucosidase preparation could enhance the protein content of wines. In order to avoid this, β-glucosidase immobilized on surface layer was entrapped in calcium alginate gel. The yield of gel entrapping was 37 % and the activity of the new β-glucosidase preparation was 1 μmol min^{-1} calcium alginate gel g^{-1}.

3.3. Modelling of food-industrial applications of α- and β-glucosidases and pectinmethylesterase

3.3.1. Flavour enrichment of wine samples by immobilized β-glucosidases

On the basis of the behaviour of immobilized β-glucosidase forms both on Acrylex C-100 support and on S-layer followed by an entrapping method in mixtures containing ethanol (15 %), it was presumed that the stability of different β-glucosidases could be enough to be used for the treatment of Muscat wines. In flavour enrichment processes wine samples were shaken with immobilized β-glucosidase preparations (200 μmol min^{-1} β-glucosidase activity in a litre of wine) for 16 h at room temperature (22-25 ^{o}C). After decantation of immobilized enzyme preparations the volatile components were extracted by a mixture of diethyl ether and pentane 2:1 then the flavour enrichment of wines was tested by gas chromatography (GC). The flavour enrichment process was characterized by a significant enhancing in the concentration of two characteristic volatile compounds: geraniol and linalool. These preliminary results suggest that both β-glucosidase preparations tested could be used for flavour enrichment of wines but at the moment they are too expensive for an industrial use.

3.3.2. Quality amelioration of food-industrial carrot preparations by the deactivation of endogenous pectinmethylesterase

The application of pectinmethylesterase in food industry was modelled by the quality amelioration of carrot preparations (a mixture modelling commercial carrot juice and carrot pieces) by the deactivation of endogenous pectinmethylesterase. The inactivation parameters of the heat and high-pressure treatments were selected on the basis of the results of the treatment of native pectinmethylesterase. It was found that both the mixture modelling commercial carrot juice and carrot pieces followed first-order kinetic models just as in the case of native pectinmethylesterase.

The sensitivity of the endogenous pectinmethylesterase content of carrot pieces to heat and high-pressure was lower than that of native pectinmethylesterase. It is supposed that the

native environment of pectinmethylesterase in carrot pieces could stabilize the enzyme. There was no combination of parameters found to make possible a considerate, fast and effective inactivation of the endogenous pectinmethylesterase content of carrot preparations.

3.3.3. Study of the activity of endogenous α- and β-glucosidases and pectinmethylesterase in raw materials of plant origin in food industry

It was studied whether the activity of endogenous α- and β-glucosidases and pectinmethylesterase in food-industrial raw materials of plant origin can be informative for their storage, transport and use. It was found that the activity of endogenous α-glucosidase was not strong enough for further studies.

β-Glucosidase and pectinmethylesterase were tested for the characterization of blanching in broccoli and cauliflower. In broccoli the original activity of β-glucosidase was 144 μmol min^{-1} broccoli g^{-1} and pectinmethylesterase was 11.44 μmol min^{-1} broccoli g^{-1}. In cauliflower the original activity of β-glucosidase was 377 μmol min^{-1} cauliflower g^{-1} and pectinmethylesterase was 3.93 μmol min^{-1} cauliflower g^{-1}. It was found that both β-glucosidase and pectinmethylesterase could be informative for the characterization of blanching and storage in deep freezer. After blanching (2-6 min, 90-98 °C) the residual activity of β-glucosidase was 20-35% (in broccoli) and 15-28% (in cauliflower) of their original activity, that was taken as hundred percent. The inactivation of β-glucosidase in both raw materials of plant origin continued during storage in deep freezer. As a result of blanching pectinmethylesterase was completely inactivated and its activity did not regenerate during storage in either broccoli or cauliflower.

I found no significant change in the activity of endogenous β-glucosidase and pectinmethylesterase in different food-industrial raw materials of plant origin (e.g. sweet almond) during their traditional storage.

3.4. Preparative-scale synthesis of surfactant *O*-alkyl-glucosides by α- and β-glucosidases catalyzed reverse hydrolytic processes

3.4.1. Optimization of reverse hydrolytic synthesis of *O*-alkyl-glucosides

For the determination of the best parameters of preparative-scale synthesis, the reverse hydrolytic process (*n*-butanol, *n*-pentanol, *n*-hexanol and cyclohexanol as both glucosyl acceptors and diluting agents, both native α- and β-glucosidase and their immobilized forms on Acrylex C-100 support) described earlier (Department of Applied Chemistry) was optimized. In

consequence of these examinations the yields of reactions catalyzed by β-glucosidase immobilized on Acrylex C-100 (153 μmol min^{-1} xerogel g^{-1}) were enhanced from 20-25 % to 40-60 %. The upscaling of the reactions was carried out in reaction mixtures of 10 % water content with six-day reaction time at room temperature. The yields of reverse hydrolytic processes catalyzed by α-glucosidase, even in immobilized form on Acrylex C-100 support were lower than 28 %. The yields were determined only by analytical methods, especially by HPTLC.

The scales of synthetic reactions were enhanced for preparative isolation and structure identification of the compounds. At first this conception failed because of the complex heterogeneity of reaction mixtures. In order to reduce heterogeneity different organic solvents as diluting agents were introduced in spite of unsuccessful earlier attempts. After a lot of unsuccessful experiments it was found that 1,2-diacetoxyethane and triacetin are suitable diluting solvents, because they can reduce the heterogeneity in the reverse hydrolytic reaction mixtures. The efficiency of these solvents was ascribed to their hydrophobic character and water solubility.

In the presence of diluting agents 1,2-diacetoxyethane and triacetin the stability values of immobilized β-glucosidase were similar to data in pure alcohols but immobilized α-glucosidase practically lost its activity. On the basis of the analytical HPTCL method the yields of synthetic reactions were 30-45 % even in the mixtures of alcohols as glucosyl acceptors and 1,2-diacetoxyethane or triacetin as diluting agents (1:9) of 10% water content at room temperature after six days. It was suggested that diluting agents 1,2-diacetoxyethane and triacetin can be useful not only for alcohols but also for other glucosyl donor compounds unsuitable for the role of solvent (e.g. phenols) for the synthesis of *O*-β-D-glucosides by reverse hydrolysis. These studies were connected with the synthesis of a new type of surfactant *O*-glucosides, alkoxyalkyl-*O*-glucosides.

The study of synthetic reactions was connected with the kinetic analysis of α- and β-glucosidase deactivation in these reactions. The results suggest first order kinetics for inactivation for immobilized α- and β-glucosidases. In the presence of diluting agents 1,2-diacetoxyethane and triacetin I found similar deactivation kinetics to kinetics data in pure alcohols for β-glucosidase immobilized on Acrylex C-100 support. The stability of native β-glucosidase was significantly higher in triacetin than in 1,2-diacetoxyethane.

3.4.2. The preparative scale synthesis of *O*-substituted-β-D-glucopyranosides by reverse hydrolysis

In the reaction mixtures containing 10 % of water content for preparative-scale syntheses the molecular ratio of glucose and hydroxyl compounds as glucosyl acceptors were 1:20 and the ratio of hydroxyl compounds and diluting agents 1,2-diacetoxyethane and triacetin were 1:9. During the preparative-scale synthesis of *O*-substituted-β-D-glucopyranosides in the case of 1,2-diacetoxyethane an *evaporation method* and in the case of triacetin solvent (because of the high boiling temperature of triacetin) an *exaction method* were used. The products were isolated by thick-layer chromatography and their structure and optical purity was controlled by ^{1}H-NMR spectra. The yields of the preparative scale syntheses of *O*-substituted-β-D-glucopyranosides were 10-25 %.

4. NEW SCIENTIFIC ACHIEVEMENTS

1. Introduction of diluting agents into reverse hydrolytic syntheses

On the basis of kinetic constants and stability (immediate deactivation and deactivation kinetics) of β-glucosidase both in native (commercially available, lyophilized) and immobilized on Acrylex C-100 support forms in alcohols of different water contents, reaction parameters were determined for testing different organic solvents as diluting agents in reverse hydrolytic processes. It was found that 1,2-diacetoxyethane and triacetin can be useful for preparative scale synthesis of *O*-alkyl-glucosides with reverse hydrolytic processes catalyzed by β-glucosidases immobilized on Acrylex C-100 support in the ratio of hydroxyl compounds and diluting agents 1:9. The stability of native β-glucosidase was enough for the synthesis only in triacetin.

2. The preparative-scale synthesis of *O*-alkyl-glucosides by reverse hydrolysis

For the preparative isolation of *O*-alkyl-glucosides from the reaction mixtures containing diluting agents new methods (*evaporation – thick-layer chromatography method* and *extraction – thick-layer chromatography method*) were elaborated. It was found that diluting agents 1,2-diacetoxyethane and triacetin are useful for glucosyl donor compounds unsuitable for the role of solvent to synthesize *O*-β-D-glucosides by reverse hydrolysis, e.g. with phenol *O*-phenyl-β-D-glucopyranoside (13 %) and with 4-nitrophenol *O*-4-nitrophenyl-β-D-glucopyranoside (11%).

3. Surface layer (S-layer) as a support for enzyme immobilization

Surface layer isolated from *Bacillus stearothermophilus* PV72 has not been used for enzyme immobilization earlier. Enzymes were immobilized on the surface layer by a special co-cross-linking with glutaraldehyde. This new method was tested for several enzymes but the yield was higher than 10 % (12.5 %) only in the case of β-glucosidase.

4. Flavour enrichment of wine samples by immobilized β-glucosidases

Modelling of food-industrial application of β-glucosidase immobilized on the surface layer was tested by its flavour modification capacity in wine samples. In order to avoid the increase in the protein content of wines, β-glucosidase immobilized on surface layer was entrapped in calcium alginate gel. The effect of this new immobilized β-glucosidase preparation was compared with β-glucosidase immobilized on Acrylex C-100 support that has not been used for the treatment of wines earlier. It was found that both β-glucosidase preparations tested could be used for flavour enrichment of wines.

5. Kinetic studies on inactivation of native pectinmethylesterase and carrot preparations

It was found that native pectinmethylesterase as model system and different carrot preparations (mixture modelling commercial carrot juice and carrot pieces) followed first-order kinetic models in the case of both heat inactivation and high-pressure treatment.

6. Characterization of changes in raw materials of plant origin during different food-industrial processes by the change in the activity of endogenous β-glucosidase and pectinmethylesterase

It was found that the inactivation of endogenous β-glucosidase and pectinmethylesterase in carrot, broccoli and cauliflower can be more characteristic for the effect of blanching and storage in deep freezer than that of peroxidase widely used for determination of the degree of blanching.

5. CONCLUSIONS AND SUGGESTIONS

- On the basis of my results I suggest an extension of the application of 1,2-diacetoxyethane and triacetin as diluting agents for other glucosidases even for other glycosidases not only in reverse hydrolytic but also in transglycosylation processes.
- It is supposed that the S-layer as support for enzyme immobilization can be used for other enzymes as well.

- I propose detailed studies of immobilized β-glucosidase both on Acrylex C-100 support and on S-layer followed by an entrapping method in flavour enrichment of different kinds of wine samples.
- On the basis of my preliminary results in heat inactivation and high-pressure treatment of different carrot preparations I suppose that the kinetic studies of endogenous pectinmethylesterase can be informative in food-industrial raw materials of plant origin after different treatments.
- Only on the basis of further studies can be established whether the changes in the activity of endogenous hydrolases, especially α- and β-glucosidases and pectinmethylesterase could be used for the characterization of storage of some food-industrial raw materials of plant origin or for the distinction of their different varieties.

6. PUBLICATIONS RELATED TO THE DISSERTATION

6.1 Publications in international journals

TERÉZ BALOGH, LÁSZLÓ BOROSS, JUDIT KOSÁRY, 2003. Novel reaction systems for the synthesis of *O*-glucosides by enzymatic reverse hydrolysis, *Tetrahedron.* 60, 679-682

TERÉZ BALOGH, CHANTAL SMOUT, BINH LY NGUYEN, ANN M. VAN LOEY, MARC E. HENDRICKX, 2004. Thermal and high-pressure inactivation kinetics of carrot pectinmethylesterase (PME): from model system to real foods, *IFSET*. 5, 429-436

BALOGH, T., TÓTH, Á., KOSÁRY, J. 2005. Immobilized β-glucosidase preparations for the treatment of wines. *Acta Alim.* 34, 203-208

BALOGH, T., KOSÁRY, J. 2004. A preparativ-scale synthesis of *O*-alkyl-β-D-lucosides by enzymatic reverse hydrolysis. *Acta Alim.* **(**under publication**)**.

6.2 Publications in home journals

KOSÁRY J., BALOGH T., KISS N., KORBÁSZ M. 2004. A lipoxigenázok izoenzim összetételének változásai a növényekben. 3. A mandula (*Prunus amygdalus*) vizsgálata biokémiai módszerekkel. *Olaj, szappan, kozmetika* 53, 11-13

6.3 Full proceedings in English

TERÉZ BALOGH, BINH LY NGUYEN, ANN VAN LOEY, CHANTAL SMOUT, MARC E. HENDRICKX, 2002. Inactivation kinetics of pectinmethylesterase in model systems and in carrot pieces. 8th PhD Symposium on Applied Biological Sciences, Gent, Belgium, 273-276.

6.4 Full proceedings in Hungarian

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2003. A β-glükozidáz inaktiválódása a reverz hidrolízis folyamán. Műszaki Kémiai Napok, Veszprém, Filka J. szerk., 355-359.

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2004. *o*-β-Glükozidok előállítása hígítószert is tartalmazó reverz hidrolitikus reakcióelegyekben. Műszaki Kémiai Napok, Veszprém, Filka J. szerk., 61-64.

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2004. Közvetlenül fogyasztásra kerülő répakészítmények eltarthatóságának növelése. Műszaki Kémiai Napok, Veszprém, Filka J. szerk., 73-77.

BALOGH TERÉZ, REZESSYNÉ DR. SZABÓ JUDIT, BOGNÁR CSABA, 2000. Funkcionális élelmiszerek tervezése és tesztelésük *in vivo* állatkísérletekben. A MÉTE XIII. Országos Tudományos Diákköri Konferenciája, Mosonmagyaróvár, Kárász Á. Szerk., 46-50.

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2001. Emulgeáló szerek szintézise biokémiai módszerekkel. Műszaki Kémiai Napok, Veszprém, Filka J. szerk., 165-169.

BALOGH TERÉZ, DR. BOROSS LÁSZLÓ, DR. KOSÁRY JUDIT, 2002. Reverz hidrolitikus reakciók eddig még nem vizsgált szerves közegben. Műszaki Kémiai Napok, Veszprém, Filka J. szerk., 255-259.

6.5 Short proceedings in international conferences

TERÉZ BALOGH, BINH LY NGUYEN, ANN VAN LOEY, MARC HENDRICKX, 2002. Effect of pH on the thermostability of carrot pectinmethylesterase, Marie Curie Workshop, Brussel, Belgium

TERÉZ BALOGH, LÁSZLÓ BOROSS, JUDIT KOSÁRY, 2002. Reverse hydrolytic activity of native β-glucosidase in an unusual, environmentally friendly solvent, Applied Biocatalysis, Como, Italy

6.6 Short proceedings in home conferences

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2001. Élelmiszer adalékok szintézise biokémiai módszerekkel. A Magyar Biokémiai Egyesület Molekuláris Biológiai Szakosztálya 6. Munkaértekezlete, Sárospatak

BALOGH TERÉZ, KOSÁRY JUDIT, 2001. Új detergensek szintézise enzimes reverz hidrolízissel. Vegyészkonferencia, Hajduszoboszló

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2003. Élelmiszeripari szempontból fontos hidrolázok enzimkinetikai vizsgálata. A Magyar Biokémiai Egyesület Molekuláris Biológiai Szakosztálya 8. Munkaértekezlete, Tihany

BALOGH TERÉZ, DR. KOSÁRY JUDIT, 2004. β-glükozidáz rögzítése felületi rétegekre. A Magyar Biokémiai Egyesület Molekuláris Biológiai Szakosztálya 9. Munkaértekezlete, Sopron

BALOGH TERÉZ, VITÉZ VERONIKA, DR. KOSÁRY JUDIT 2004. Új enzimek tesztelése az előfőzés jellemzésére. Nemzetközi Élelmiszeripari konferencia, Szeged