COST 928
WG 1-3 Meeting

Norwich, 3-4 April 2008

Table of contents

POSTER PRESENTATIONS

ORAL PRESENTATIONS

O1. RECOMBINANT EXPRESSION AND CHARACTERIZATION OF A NAD(P)(H) PHOSPHODIESTERASE OF WHEAT THAT MAY IMPACT GLUTEN NETWORK FORMATION DURING FLOUR PROCESSING

I.J Joye.* K. Fierens and J.A. Delcour

Laboratory of Food Chemistry and Biochemistry, KULeuven, Leuven, Belgium

Gluten proteins confer the unique property of dough making ability to wheat flour as they form what is referred to as a gluten network. Thiol-disulfide inter-change reactions are very important for the properties of such network. In industrial practice, the balance between thiol and disulfide moieties is impacted by redox reagents such as ascorbic acid. The literature also describes positive effects of the natural electron transfer agents nicotinamide coenzymes NAD(P)(H) on breadmaking quality. Parmentier (2004) reported coenzyme degradation by NAD(H) phosphodiesterases present in wheat. As such, these enzymes possibly influence wheat properties during processing and therefore thorough characterization is needed.

Based on protein sequences of known mammalian NAD(P)(H) hydrolysing enzymes, a DNA sequence coding for a putative nucleotide pyrophosphatase/phosphodiesterase was assembled using wheat EST databases. The DNA sequence was isolated from wheat RNA by RT-PCR. It codes for a protein with a theoretical molecular mass and pI of 52 kDa and 5.68, respectively. The wheat enzyme contains a predicted *N*-terminal trans-membrane domain and several putative *N*-glycosylation sites. Recombinant ex-pression was performed using the yeast *Pichia pastoris* as expression host. The recombinant enzyme was purified and biochemically characterized. Molecular mass and pI were verified. pH- and T-stabilities and –optima were determined using *p*-nitrophenyl thymidine monophosphate as substrate. Furthermore, recombinant wheat phosphodiesterase is active against NAD(P)(H). Overall, we now have a well-characterized wheat NAD(P)(H) phosphodiesterase. Future work will point out its importance in gluten network formation.

Reference

Parmentier, S. (2004). Enzymatic regeneration of coenzymes in dough systems. PhD thesis, U.Gent, Belgium.

O2. SIMULTANEOUS PRODUCTION OF THERMOSTABLE ENDO-BETA-1,4 XYLANASE AND ALDOPENTAOURONIC ACID DURING GROWTH OF *THERMOMYCES LANUGINOSUS* ON GLUCURONOXYLAN

Vladimír Puchart and Peter Biely*

Institute of Chemistry, Slovak Academy of Sciences, SK 845 38 Bratislava, Slovak Republic

Two strains of the thermophilic fungus Thermomyces lanuginosius, IMI 84400 and IMI 96213, were shown to secrete glycoside hydrolase family 11 endo-beta-1,4 xylanase and simultaneously accumulate an acidic pentasaccharide in the medium when grown on beech-wood glucuronoxylan. The oligosaccharide was purified using a combination of anion exchange and gel permeation chromatographies. The structure of the purified unconsumed glucuronoxylan fragment has been established as aldopentaouronic acid MeGlcA3(upper index)Xyl4(lower index) by a combination of NMR spectroscopy and enzyme digestion with exo-glycosidases. The accumulation of the aldouronic acid represents an evidence that the fungus does not produce alpha-glucuronidase and that acidic fragments of glucuronoxylan are not transported into the cells.

O3. DIFERULATES IN PLANT CELL WALLS AND AND ENZYME CATALYSIS

*J. Agger**

O4. HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY OF ARABINOXYLAN DIGESTS: TRAPS AND CHALLENGES

L. E. Rasmussen* and A.S. Mayer

BioProcess Engineering, Dept. of Chemical Engineering, DTU, Kgs. Lyngby, DK
Email: ler@kt.dtu.dk.

Efficient enzymatic modification and degradation of natural plant cell wall polymers have significant implications in food, biofuel and paper processing and may open up for novel biological production platforms in various industries. In the production and application of plant polymers, High Performance Size-Exclusion Chromatography (HPSEC) is a powerful technique for analyzing the molecular weight and the molecular weight distribution to characterize polymers in solution. In this work the objective is to evaluate the influence of specific parameters on the chromatographic profile obtained by HPSEC with special focus on analyzing enzymatic degradation of water insoluble arabinoxylan catalyzed by a xylanase. The hypothesis is that it is possible to optimize each of the parameters in order to improve the chromatographic profile. Assessment of the enzymatic degradation of WUAX by a xylanase is accomplished by use of High Performance Size Exclusion Chromatography (HPSEC) to determine the molecular weight and the molecular weight distribution of the hydrolysates. Different type and concentration of the mobile phase have been used. The experimental data have revealed that depending on the type and concentration of mobile phase and positive and negative peaks were obtained by injecting the sample solution with varying retention times and area. These peaks interfere with the chromatographic profile of arabinoxylan digests and therefore have to be eliminated. Furthermore, interactions between the eluted molecules and the stationary phase modify the elution order of compounds with definite molecular weights, which results in secondary mechanisms accompanying the main "ideal" SEC separation mechanism. It is important to identify the mode of action when using HPSEC and either eliminate or take advantage of it when useful. In both cases the appropriate selection of the mobile phase composition is needed.

Keywords: size-exclusion, secondary effects, arabinoxylan, xylanase.

O5. REMOVAL OF N-TERMINAL PEPTIDES FROM β-LACTOGLOBULIN BY PROTEOLYTIC CONTAMINANTS IN TYROSINASE AND LACCASE PREPARATIONS INFLUENCES ITS DIGESTION BY PEPSIN

Dragana Stanic, Natalija Polovic, Milica Popovic, Lidija Burazer, Ratko Jankov and Tanja Cirkovic Velickovic*

Faculty of Chemistry, Department of Biochemistry, Belgrade, Serbia
Institute of Immunology and Virology, Torlak, Belgrade, Serbia

β-lactoglobulin (BLG) is an important nutrient of the dairy products. It also represents a serious health risk in patients allergic to milk. In this study we examined the effect of mushroom tyrosinase and laccase from *Trametes versicolor* on the BLG. The BLG was exposed to mushroom tyrosinase or laccase and the obtained products were analyzed by SDS PAGE and immunoblotting with anti-BLG specific antibodies. The reaction mixture was HPLC purified and analyzed by ESI MS. The treated BLG was exposed to the simulated conditions of the gastrointestinal tract and the reaction was monitored by SDS PAGE. The ESI MS analysis showed that both forms of BLG, BLG A and BLG B, undergone the same change in the presence of laccase. The N-terminal pentapeptid was removed by the laccase treatment from both forms. The tyrosinase treatment romoves the N-terminal tetrapeptid. The truncated forms bind BLG specific antibodies, but became more susceptible to pepsin digestion.

In this study we have shown that BLG, although not a good substrate for oxidases is a good substrate for proteolytic contaminants in commercial laccase and tyrosinase preparations. The BLG, known to be extremely resistant to digestion, in the presence of these enzymes became truncated for a discrete peptide fragment from the N-terminal and thereby more susceptible to digestion by digestive enzymes.

O6. POLYFUNCTIONAL COMPOUNDS AS INHIBITORS OF MONOAMINEOXIDASE

Michaela Dina Stanescu[1]*, Ovidiu Oprea[2] and Ioana Jitaru[2]

[1]"Aurel Vlaicu" University of Arad, Department of Chemical and Biological Sciences, 3 E. Dragoi str, 310330 Arad, Romania
[2]"Politehnica" University, Department of Inorganic Chemistry, 1Polizu str., Bucharest, Romania

During the process of fermentation of foods and beverages biogenic amines are produced by lactic acid bacteria from amino acids by decarboxilation. These compounds are found in: cheese, wine, beer, sauerkraut, fishery products and aged meat. They are aliphatic, alicyclic or heterocyclic amines of low molecular mass and their presence in high amount in foods is associated with food deterioration as well as toxicity. In non-fermented foods, the biogenic amines appear as a result of undesirable microbial activity. In plants, biogenic amines have been implicated in many cell processes, like membrane stability, synthesis of nucleic acids and proteins, division and differentiation, pH regulation and thermic or osmotic stress responses, and delay in senescence.

The enzyme regulating the biogenic amine content is Monoamine oxidase, enzyme based on flavin-adenine dinucleotide as coenzyme. It transforms the biogenic amines in aldehydes which are subsequently oxidized to carboxylic acid and eliminated. The control of the enzyme activity is insured by inhibitors. Syntheses of such inhibitors are presented starting from α-Chloroacetophenone and different amines, as well as the preparation of the coordination compounds of these keto-amines. The properties of the new synthesized compounds are presented.

References

Stanescu, M.D., Jitaru, I., Oprea, O., Jinga, O. Coordination Compounds of a Potential Polydentate Ligand 3. Co(II) and Ni(II) Complexes of an α-Aminoketone, *Roum.Biotechnol.Lett.*, 1998, *3,* 83-89.

Stanescu, M.D., Jitaru, I., Oprea, O., Negreanu-Pirjol, T., Jinga, O.A. Biological Activity Studies of Complex Compounds with ligands of IMAO Type. *Proc 12th Romanian Int Conf Chemistry and Chemical Engineering 13-15 September 2001*, *BUCHAREST Romania, Vol Inorganic Chemistry,* 396-399.

Stanescu, M.D., Jitaru, I., Oprea, O.A.. New Potential Monoamineoxidase Inhibitors. *Book of Abstracts of 1st European Congress of Chemistry, 27-31 August 2006, BUDAPEST, HUNGARY*

O7. INTERCULTIVAR DIFFERENCES IN POLYMORPHIC FAMILIES OF THREE TYPES OF XYLANASE INHIBITORS IN THE WHEAT GRAIN PROTEOME

Evi Croes*, Kurt Gebruers, Johan Robben, Erwin Witters, Jan A. Delcour and Christophe M. Courtin

Laboratory of Food Chemistry and Biochemistry, K.U. Leuven, Belgium
Email: evi.croes@biw.kuleuven.be

Cereals contain proteinaceous inhibitors of endo-β-1,4-xylanases. As xylanase inhibitors (XIs) are active against xylanases of microbial origin and do not interact with plant xylanases, they are believed to act as a defensive barrier against phytopathogenic attack. Moreover, their importance in reducing the activity of added microbial xylanases in wheat-based food processes has convincingly been demonstrated.

Affinity-purification and 2D-gel electrophoresis (2-DE) of the three currently known types of XIs, i.e. *Triticum aestivum* L. xylanase inhibitor (TAXI), xylanase inhibiting protein (XIP) and thaumatin-like xylanase inhibitor (TLXI), revealed the existence of polymorphic protein families. Using mass spectrometric analysis different genetic variants could be distinguished, while further variation could be ascribed to post-translational modifications.

Next, TAXI-, XIP-, and TLXI-type XIs were traced in complex but reproducible, high-resolution 2-DE profiles of wheat soluble seed proteins using immunoblotting and probing with antibodies. To quantify and identify biologically relevant cultivar-dependent expression differences 2-DE and MS/MS were applied after fluorescent labelling (DIGE) of protein samples. Six wheat cultivars were selected based on differences in XI contents. Univariate and multivariate statistical analysis were used to unravel significant expression changes in XIs and to find similarities in protein patterns between XI and non-XI proteins.

Extending our knowledge on XIs will undoubtedly lead to a better understanding of their physiological role and, from a processing point of view, will result in a more profound insight in their function in bread-making and other biotechnological applications.

O8. PARTIAL PURIFIED TRITICALE ALBUMINS AND THEIR INHIBITION ACTIVITY AGAINST FUNGAL ENDOXYLANASES FROM *TRICHODERMA REESEI* AND *ASPERGILLUS ORYZAE*

D. Vidmantiene[1], G. Juodeikiene[1], V. Narbutaite[1], H. Sørensen[2], S. Sørensen[2] and C. Bjergegaard[2]

[1]Kaunas University of Technology, Kaunas, Lithuania
[2]University of Copenhagen, Denmark

The variation in the activity of triticale kernel-associated endoxylanases was investigated using six winter triticale varieties grown in 2006 with falling numbers on an average of 64 s. The study includes a partial purification of proteinaceous triticale inhibitors by Flash chromatography using gradient (pH 3.0-8.6) elution. The determination of the molecular size and pI by SDS-PAGE and isoelectric focusing was performed respectively. The determination of the molecular size and pI was performed by SDS-PAGE and isoelectric focusing respectively. The inhibition activity of triticale albumins against fungal endoxylanases from *Trichoderma reesei* and *Aspergillus oryzae* was analyzed. The apparent endoxylanase activity was quantified with the dinitrosalicylic acid assay based on the measurement of reducing sugars released from Birchwood xylan.

The apparent endoxylanase activity between the different triticale kernels varied from 1.3 to 6.7nkat/g. The endoxylanase activity was largely depended on the variety as well as the levels of the endoxylanase proteins with inhibitory activities that were essential influenced by genetic factors.

Endoxylanase inhibition activity was detected in the albumin fractions of triticale by elution with 0.3 M NaCl solution (pH 8.3). The inhibitor(s) were found as to have molecular weights of about 11; 30,6;18 and 40 kDa by pI‘s between 8.7 and 9.3.

The microbial endoxylanase from *A. oryzae* was found to be more active and sensitive to soluble birch wood xylan than that of *T. reesei* xylanase. However, the *A. oryzae* xylanase was much more sensitive to inhibition by triticale inhibitors.

The results obtained may be useful in explaining the differences in functionality of different endoxylanases in biotechnological applications as well as in screening of commercial enzymes for the applications in triticale-based processes.

Keywords: triticale, albumins, purification, endoxylanase, inhibition**.**

O9. STRUCTURAL BASIS FOR PROTEIN RECOGNITION BY THIOREDOXIN AND QUANTITATIVE DISULFIDE PROTEOMICS

K. Maeda[1], P. Hägglund[1], A. Henriksen[2], A. Shahpiri[1], B.C. Bønsager[1], C. Finnie[1] and B. Svensson[1*]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark
[2]Biostructure Group, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

Thioredoxin (Trx) is a ubiquitous redox protein involved in key life processes and reduces protein disulfide bonds providing reducing equivalents or modulating enzyme activities. Trx has been extensively studied for the past 30 years but target protein recognition is still poorly understood. We study the molecular mechanisms of two h-type isoforms HvTrxh1 and HvTrxh2 identified in barley seeds and identified several target proteins using proteomics techniques. We have recently established a quantitative proteome analysis of the extent of Trx (or other "reagents") protein disulfide reduction and apply this to identify status for individual protein disulfides in cereals[1], such as e.g. the C144-C148-disulfide in barley α-amylase/subtilisin inhibitor (BASI). We determined the first 3D structure of a Trx-target protein complex (HvTrxh2-BASI) as a disulfide bonded reaction intermediate[2]. The structure reveals interactions between conserved hydrophobic motifs in Trx and a sequence of BASI residues through backbone-backbone hydrogen bonds and van der Waals' contacts. This binding mode indicates that recognition of features around protein disulfides are important for Trx target specificity. The quantitative disulfide bond proteome analysis shall be validated regarding structural motifs. The new information has impact on cereal food technology in particular since the homologous NADP thioredoxin reductase is now available[3].

References

1. Maeda, K., Finnie, C. & Svensson, B. *Proteomics* 5, 1634-1644 (2005).
2. Maeda K., Hägglund P., Finnie C., Svensson B. & Henriksen A. (2006) *Structure* 14, 1701-1710.
3. Shapiri A, Svensson B, Finnie C. (2008) *Plant Physiol.* 146, 789-799.

Support from the Danish Research Councils for Technology and Production Sciences, and Natural Science, LMC, Ph.D. stipends from DTU (KM, BCB) and the Iranian government (AS) are gratefully acknowledged.

O10. CHARACTERIZATION AND MODIFICATION OF RYE FLAVOUR BY ENZYMATIC MEANS

Emilia Selinheimo, Raija-Liisa Heiniö, Pekka Lehtinen, Anu Kaukovirta-Norja, Kaisa Poutanen and Johanna Buchert

VTT, Technical research Centre of Finland, POB 1000, FI-02044 VTT, finland

Even if foods made of cereal whole grain are considered healthy, certain intrinsic flavours of the whole grains are often not regarded attractive by consumers. In particular, intensive and bitter flavour of rye, which is expected to be caused by specific phenolic compounds and small peptides, can form obstacles for its use. The flavour of cereal foods could be modified by different enzymatic treatments. However, bioprocessing of flavour of whole grain cereals can be challenging, since, due to the complexity of grain structure, flavour compounds are not uniformly distributed in the kernel. In rye grain, the innermost endospermic part is considered mild-tasting, whereas the outermost bran fraction, which also contains significant amounts of bioactive compounds, is perceived as bitter. Hence, understanding detailed chemistry of cereal whole grains is essential for the controlled enzymatic processing of the presumed flavour attributes.

The aim of the study was to increase understanding of correlations between the chemistry and the characteristics of perceived flavour of rye, and to develop enzymatic techniques for targeted flavour modification of rye-derived foods. Hydrolytic enzymes (such as, proteases, lipases and xylanases) and crosslinking enzymes (such as, peroxidase, laccase, tyrosinase and transglutaminase) were used to selectively modify non-volatile compounds of rye bran. Flour-water suspensions of the bran were prepared and treated with the enzymes, after which representational chemical analyses and sensory descriptive profiling of the samples were performed. It was observed that the flavour of rye bran was notably altered by the different enzymatic treatments, and the hydrolytic enzymes also decreased the viscosity of the rye suspension. It was found out that only the peroxidase treatment seemed to decrease the rye bitterness to some extent. On the other hand, proteases and lipases, in particular, increased the bitter flavour and irritant aftertaste of rye, suggesting both peptides and lipids to have a distinctive role in rye flavour formation. Furthermore, different substrate specificities of the enzymes seemed to have individual impacts on the flavour modification of rye.

O11. OPTIMISATION OF THE ANTIOXIDANT CAPACITY DURING SOYBEAN FERMENTATION WITH FUNGI

Severino S Pandiella

School of Chemical Engineering and Analytical Sciences, The University of Manchester, Sackville Street, POB 88, Manchester M60 1QD, UK

Soybeans are not only sources for basic nutritional compounds such as protein and oil but also of phytochemicals like isoflavones and phenolic acids with antioxidant activity. This activity is affected by the preparation and treatment of the beans and is also significantly higher after fermentation with fungi due to the action of the hydrolytic enzymes. In particular, β-glucosidase is responsible for the transformation of glucosides into the unconjugate forms which results in the enhancement of the antioxidant properties.

The objective of this study is to investigate the □-glucosidase activity developed through fungal growth in relation the total phenolics content and DPPH (2,2-diphenyl-1-picrylhydrazyl) produced during fermentation of soybean with Aspergillus oryzae and Aspergillus awamori over an 8-day incubation period. A mathematical model has also been used to optimize the antioxidant production and extrantion. A. oryzae showed in all cases a higher yield of antioxidant production. The mathematical model used confirmed that soybean fermentation with A. oryzae for 5 days was the optimum for phenolics and DPPH production.

O12. IMPACT OF PREBIOTICS AND HEMICELLULASES ON THE QUALITY PROFILES OF WHITE SOURDOUGH BREAD

A. Miezeliene and G. Alencikiene

Food institute of Kaunas Technological University, Taikos pr. 92, LT-51180, Kaunas, Lithuania

Prebiotics have attracted increasing attention in recent years as functional ingredients for different food products which ferment in the colon due to specific health-promoting bacteria. The objective of the present study were, (a) to produce white sourdough bread enriched with prebiotic (acacia gum), (b) to examine the influence of different doses of prebiotic on rheological properties of dough and quality attributes (specific volume, sensory and textural profiles) of resulting bread, (c) to examine the integrated impact of prebiotic and exogenous xylanase on quality attributes of dough and baked goods. A purified 1,4-beta xylanase produced by a genetically modified strain of *Aspergillus oryzae* was used for improving baking properties of mixture of wheat-rye flour. A sensory evaluation was carried out with a trained panel of 8 assessors consisting of KTU Food Institute staff using descriptive analysis method. Rheological attributes of the dough and bread texture were evaluated by Universal testing Machine Instron 3343 (Instron Engineering Group, High Wycombe, UK).

O13. MICROSTRUCTURAL CHANGES OF CONCENTRATED TOMATO SUSPENSIONS ON HOMOGENISATION AND SUBSEQUENT SHEARING

Elena Bayod and Eva Tornberg*

Department of Food Technology, Engineering and Nutrition, Lund University, POB 124, SE- 22201, Lund, Sweden
Email:eva.tornberg@food.lth.se

The microstructural properties of tomato suspensions have been investigated on homogenisation, and subsequent shearing at three tomato paste concentrations of 10, 30, and 40 %. The microstructural changes were recorded, using particle size analysis (laser light diffraction), light microscopy and image analysis (at 10 % paste concentration), volume fraction of the suspension and low amplitude oscillatory rheology. Before homogenization the tomato samples consist of more or less spherical and deformable particles (i.e. the tomato cells). Homogenization not only decreases the size of the particles, but it also creates a new network structure. Subsequent shearing of the suspensions, causes a disruption of the network, especially in the most homogenised samples, giving rise to the aggregation of the particles into densely packed flocs, which are easily oriented in the shearing direction. Image analysis of the pictures suggests a fractal structure. That fractal behaviour was confirmed by the rheological data, which followed the equation $G' = \alpha\phi^{1/(3-D_f)}$, where the elastic modulus (G') is a function of the volume fraction (ø), the fractal number (D_f) and a constant (α) which depends on the particle size, aspect ratio, the maximum packing and also the fine to coarse ratio (f/c). All suspensions exhibit a gel-like behaviour, with G'>>G''.

O14. ENZYME SACCHARIFICATION OF ARABINOXYLAN IN PRE-TREATED WHEAT BRAN

Laurice Pouvreau*, Henk Schols

Laboratory of Food Chemistry, Wageningen University, The Netherlands

The production of fuel bio-ethanol from cellulosic biomass is studied worldwide in order to substitute the use of petrochemical fuels. This process is generally associated with a certain type of biomass-pretreatment such as heat and acid. Many studies have been performed on pretreatments at elevated temperature and/or at low pHs on hard wood, but very little is known on the effect of such pretreatment on soft wood like wheat bran.

Wheat bran is an agricultural by-product, which is mainly composed of starch (16-18%), cellulose (15-17%), hemicellulose (18–25%), lignin (14–16%) and protein (13-15%). A combined severity factor was used to express the pretreatment including the temperature (120 up to 180°C), the time of incubation (10min) and the pH.

The pretreatment of wheat bran is studied by determining the relation between severity factor and the composition of the fractions obtained after pretreatment. The susceptibility of cellulose and hemicellulose towards enzymatic hydrolysis after pretreatment is also discussed.

POSTER PRESENTATIONS

P1. PYRANOSE DEHYDROGENASE FROM *AGARICUS MELEAGRIS*: CHARACTERIZATION AND APPLICATION IN CARBOHYDRATE CONVERSIONS

Christoph Sygmund, Roman Kittl, Jindrich Volc, Petr Halada, Dietmar Haltrich and Clemens K. Peterbauer*

Dept. of Food Sciences and Technology, Universität f. Bodenkultur Wien

Pyranose dehydrogenase (PDH) of the mushroom *Agaricus meleagris* was purified from mycelial culture media to substantial homogeneity using ion-exchange and hydrophobic interaction chromatography. The native enzyme is a monomeric polypeptide with a molecular mass of 66,547 Da containing ~ 7% carbohydrate and covalently bound flavin adenine dinucleotide. The enzyme exhibited a broad sugar substrate tolerance, oxidizing different aldopyranoses to the corresponding C-2 or C-2,3 (di)dehydro sugars. Preferred electron donors with the highest k_{cat}/K_m values were major sugar constituents of cellulose and hemicellulose, namely D-glucose, D-galactose, L-arabinose, D-xylose and cellobiose. This indicates a possible physiological role of the enzyme in lignocellulose breakdown. PDH showed no detectable activity with oxygen, alternative electron acceptors are various substituted benzoquinones and complexed metal ions, with the ferricenium ion and the benzoquinone imine 2,6-dichloroindophenole displaying the highest k_{cat}/K_m. The enzyme catalyzed in up to 95% yields the regiospecific conversion of D-galactose to 2-dehydro-D-galactose, an intermediate in a possible biotechnologically interesting process for redox isomerization of D-galactose to the prebiotic sugar D-tagatose. An analogous isomerization of lactose to lactulose is also conceivable.

P2. PECTINASES FOR IMPROVING ANTIOXIDANT FRUIT JUICE CONTENT

Dana Gina Radu & Michaela Dina Stanescu

"Aurel Vlaicu" University of Arad, Department of Chemical and Biological Sciences, 3 E. Dragoi str, 310330 Arad, Romania

The high antioxidant content of berry juices provides possible health benefits such as reduction of coronary heart disease, improved visual acuity, anti-viral and anti-cancer activity. Our objective was to determine the influence of different commercial pectolytic enzymes upon the fruits cell wall maceration, during juice production and the consequences of the enzymatic treatment upon the flavonoids extraction.

The range of total anthocyanin content and total flavonoids content in juices obtained from diverse cultivar of blackberries *(Rubus hirtus W. et. K.)* and raspberries (*Rubus idaeus L.*) were assessed. Although improvement of the content in the useful antioxidant compounds was observed for all samples treated with pectolytic enzymes, compared to the blank. Some commercial pectolytic enzymes are more efficient than other, because of their secondary activities (hemicellulasic and arabanasic) favoring the disaggregation of the skin wall structure and extraction of the antioxidants. Simultaneously, a more vivid and appealing color, an important feature of the fruit juices and an enhanced fresh fruit flavor are achieved.

References

1. Bermudez-Soto, M. J., Tomás-Barberan, F. A., Evaluation of commercial red fruit juice concentrates as ingredients for antoxidant functional juices, *Eur. Food Res Technol.,* 2004*, 219,* 133-141.
2. Bonvehí, J. S., Torrentó, M. S., and Lorente, E. C., Evaluation of polyphenolic and flavonoid compounds in honeybee-collected pollen produced in Spain, *J. Agric. Food Chem.,* 2001*, 49,* 1848-1853.
3. Fan-Chiang H-J., and Wrolstad, R. E., Anthocyanin pigment composition of blackberries, *J. Food Sci.,* 2005*, 70 (3),* C198-C202.
4. Marinova D., Ribarova F., Atananasova M., Total phenolics and total flavonoids in bulgarian fruits and vegetables, *J. Univ.Chem. Techn. Metal*., 2005, 40 *(3)*, 255-260.

P3. ACTION OF GH10 AND GH11 ENDOXYLANASES ON P-NITROPHENYL-B-D-XYLOSIDES

Maija Tenkanen

University of Helsinki, POB 56, FI-00014 University of Helsinki, Finland

Endo-β-D-xylanases (EC 3.2.1.8) are abundant enzymes randomly cutting xylan chains. They are currently classified into six different glycoside hydrolase families. Most endoxylanases belong in the families GH10 and GH11 whose members possess clearly distinctive catalytic properties. The xylooligosaccharides resulting from the activity of GH10 xylanases are shorter than those produced by GH11 xylanases. GH11 endoxylanases are hindered by the presence of side groups, whereas GH10 endoxylanases are able to act near the substitution. However, both families contain enzymes with slightly diverse catalytic sites, resulting in further small differences in their hydrolytic performance. The catalytic versatility of GH10 endoxylanases is also shown by the ability to hydrolyse small glycoside derivatives such as p-nitrophenyl- β-D-cellobioside, and in same cases even p-nitrophenyl- β-D-xylopyranoside, which are typical substrates for cellobiohydrolases and beta-xylosidases, respectively. Recently nitrophenyl-β-D-xylosides have been synthesised for simple chromogenic substrates for endoxylanases. The action of three GH10 (*Aspergillus aculeatus, Aspergillus oryzae, Nonomurea flexuosa*) and three GH11 (*Trichoderma reesei* XYN I and XYN II, *Nonomurea flexuosa*) endoxylanases towards p-nitrophenyl-β-D-xylose, and ortho and para isomers of nitropehyl-β-D-xylobiose and -xylotriose was compared in the present work.

Acknowledgements
Anna Kulminskaya is thanked for the nitrophenyl-β-D-xylosides, Ossi Turunen and Novozymes A/S for the *N. flexuosa* and *A. acuelatus* endoxylanases, respectively, Essi Harju for the skilful technical work, and the Academy of Finland for the financial support.

P4. MODE OF ACTION OF GLYCOSIDE HYDROLASE FAMILY 5 ENDO-B-1,4-XYLANASE FROM *ERWINIA CHRYSANTHEMI*

Mária Vršanská*, Katarína Kolenová, Vladimír Puchart and Peter Biely

Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia

The mode of action of xylanase A (Xyn A) from a phytopathogenic bacterium, *Erwinia chrysanthemi*, classified in GH 5, was investigated on xylooligosaccharides and polysaccharides using TLC, MALDI-TOF MS and enzyme treatment with exoglycosidases. The hydrolytic action of Xyn A was found to be absolutely dependent on the presence of 4-*O*-methyl-*D*-glucuronosyl (MeGlcA) side residues in both oligosaccharides and polysaccharides. As a rule, the enzyme attacked the second glycosidic linkage following the MeGlcA branch towards the reducing end. Depending on the distribution of MeGlcA residues on the glucuronoxylan main chain, the enzyme generating series of shorter and longer aldouronic acids of backbone polymerization degree 3-14, in which the MeGlcA is linked exclusively to the second xylopyranosyl residue from the reducing end. Upon incubation with β-xylosidase, all acidic hydrolysis products of acidic oligosaccharides and hardwood glucuronoxylans were converted to aldotriouronic acid, $MeGlcA^2Xyl_2$. In agreement with this mode of action, xylose and unsubstituted oligosaccharides were essentially absent in the hydrolysates. The *E. chrysanthemi* xylanase A thus appears to be an excellent biocatalyst for the production of large acidic oligosaccharides from glucuronoxylans as well as an invaluable tool for determination of the distribution of MeGlcA residues along the main chain of this major plant hemicellulose.

References

Vršanská, M. et al. (2007) FEBS J. 274, 1666-1677