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Cereal $n$-glycoproteins Enrichment by Lectin Affinity Monolithic Chromatography

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Research of cereal glycoproteins is important for understanding of their functional properties, their role during technological processing of cereals and it can serve as a valuable tool for the detection of cereal allergens. The main intention of this study was the screening of profile of water-soluble glycoproteins present in barley (Hordeum vulgare), wheat durum (Triticum durum) and spelt (Triticum spelta). Lectin monolithic HPLC column was used for rapid and effective enrichment of glycoprotein fractions. Captured glycoproteins were electrophoretically separated and analyzed by MALDI-TOF MS. Presented procedure resulted in identification of a group of $N$-glycoprotein candidates with affinity to lectin concanavalin A (ConA). Such molecules could have, among others, an allergenic potential. Majority of captured low-molecular-weight glycoproteins belong to alpha amylase/trypsin inhibitors family. However, most of the higher-molecular-weight proteins identified in lectin bound fractions have not been described as glycoproteins yet. Obtained results improved the knowledge about (glyco)protein content in cereal grain. The connection of lectin HPLC-GE-MS was proved as a convenient strategy for identification of cereal glycoproteins. Suggested method is universal and can be applied for various cereals and food-stuffs.

Keywords: barley, wheat, glycoprotein, mass spectrometry, lectin chromatography

Introduction

Proteins and glycoproteins

Proteomics is one of the most important areas of biological research because proteins belong to the most significant molecules in cells. They serve as antibodies, enzymes or hormones; and are involved in the structural, storage or transport functions. Proteins are also important in biotechnology and industries (e.g. food chemistry, pharmacy) (Pandey and Mann 2000). Most of the proteins are posttranslationally modified. One of the most abundant modifications is glycosylation. There are two main categories of glycoproteins: $N$- and $O$-glycoproteins. In $N$-glycoproteins, glycans are attached to the amine group of asparagine. $O$-glycosidic linkage is through the hydroxyl of serine, threonine or hydroxylysine (Robyt 1998). Glycoproteins have very important and multiple functions in living organisms. Glycans serve as molecular determinants for recognition by the cells, the mi-
croorganisms, or other biological molecules. The carbohydrate moieties are involved in stabilization of protein conformation, in the translocation of proteins through membranes, or in the protection of proteins against protease hydrolysis. Glycosylation can alter the thermal stability, viscosity or isoelectric point (pI) of proteins (Robyt 1998). Since glycans are large and hydrophilic, they can also affect protein–protein interactions and influence the solubility of a protein (Rudd and Dwek 1997).

**Cereals**

Cereals (wheat, rye, barley, oats, maize, and rice) are used as human food, animal feed (mainly for livestock and poultry) and industrially in the production of a wide range of commodities (e.g. glucose, adhesives, oils, alcohols). Barley is the fourth most important cereal crop in the world. In food industry, barley grains are used for the production of peeled barley, alcohol and particularly malt for the production of beer and whisky. Knowledge of barley grain composition is important for the brewing industry, human and animal nutrition, plant breeding, or cultivars identification. Wheat, as one of the major cereals, is the staple source of flour for bread, semolina, pasta, noodles, biscuits, and other confectionary products. It is also used for the production of alcoholic beverages. There are many species of wheat. Three of the major cultivated ones are common wheat (*Triticum aestivum*), durum (*Triticum durum*) and spelt (*Triticum spelta*). Common wheat is used mainly for bakery purposes. Durum is suitable cereal for the production of pasta products with high quality. Pasta, bread and related products made of this kind of wheat have average or higher protein content (Dukic et al. 2008). Spelt (*Triticum spelta*) is an ancient chaffy European subspecies of common wheat (*Triticum aestivum*) with undemanding requirements for growing. In comparison of common wheat, the disadvantage of spelt is its lower yield and the fact that spelt is a hulled grain thus a dehulling step prior to milling is necessary. For many years, spelt cultivation declined. Recently spelt has undergone a renaissance probably due to a fact that spelt has valuable nutritional qualities differing from those of common wheat – higher protein content – thanks to higher portion of aleuron layer, higher lipid and Mg, P, Fe, Cu and Zn contents.

**Cereal glycoproteins**

Glycoproteins were studied, e.g. in barley (Soerensen et al. 1986; Laštovičková et al. 2011), wheat (Breddam et al. 1987) or rice (Park et al. 2013). The special attention in research is concentrated to analysis of cereal allergenic glycoproteins. Snegaroff et al. (2013) investigated barley gamma 3-hordein by using immunochemical and MS methods. N-glycosylation was found at an atypical Asn-Leu-Cys consensus sequence (Snegaroff et al. 2013). Hiemori et al. (2010) and Kimoto et al. (2009) confirmed that the major allergens responsible for bakers’ asthma belong to the water/salt-soluble protein fraction of wheat. Some glycoproteins with molecular weight in the 12–16 kDa range from alpha-amylase/trypsin inhibitor family are confirmed allergens found in wheat and barley flour (Sanchez-Monge et al. 1992; Perrocheau et al. 2005).
Analysis of glycoproteins

The recent instrumental advances in MS progress in miniaturized technologies for sample handling and enrichment and connection of MS with highly sophisticated separation techniques allow to introduce the (glyco)proteomics as a powerful tool for characterization of (glyco)proteins including the determination of the exact molecular masses of proteins, the identification of their new isoforms and description of their posttranslational modifications (Lazar et al. 2011). Since different glycoproteins occur in particular biological samples in large concentration range, only highly sensitive techniques can be used for glycoproteomic analysis. Protocols, methodologies and technologies for characterization of glycoproteins including various combinations of different kind of LC, CE and MS techniques have been published in reviews (Lazar et al. 2011). MS-based applications of “Omic” sciences (proteomics, peptidomics, glycomics) for analysis of (glyco)proteins in food products have been summarized, e.g. by Picariello et al. (2012).

In this work, we described an effective analytical protocol for screening of total N-glycoproteins “pool” in cereal materials. Water-soluble proteins isolated from durum wheat, spelt wheat and barley were subjected to lectin HPLC enrichment, GE separation, MS detection and consequential bioinformatic analysis.

Materials and Methods

Chemicals and samples

All the chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany), trypsin (bovine) and chymotrypsin were obtained from Roche Diagnostics (Mannheim, Germany). ZipTip C18 pipette tips were purchased from Merck Millipore (Billerica, MA, USA) and alpha-cyano-4-hydroxycinnamic acid was purchased from LaserBio Labs (Sophia-Antipolis Cedex, France). Ready Tris–HCl gels (4–20%, 8.6 × 6.8 cm) were purchased from Bio-Rad (CA, USA). Barley samples were provided by the Research Institute of Brewing and Malting (Brno, Czech Republic). Flour of durum and spelt was purchased in a shop with healthy products.

Protein extraction

Cereal flour (50 mg) was mixed with 1.0 mL deionized water and shaken for 30 min (at room temperature). The suspension was filtered using a 0.45 µm PVDF microfilter (Millipore Corporation, Prague, Czech Republic) and lyophilized.

Lectin affinity HPLC column

Lyophilized protein extracts were resuspended in an equilibration buffer (mobile phase A) for affinity chromatography (see below), sonicated and filtered using a 0.45 µm PVDF microfilter. The separations of cereal protein extracts were performed on concanavalin A
HPLC column (ProSwift® ConA-1S monolith column, 50 × 5 mm; Thermo Scientific Dionex, Sunnyvale, USA).

Mobile phases: A) 50 mM sodium acetate, 0.2 M sodium chloride, 1 mM calcium chloride, 1 mM magnesium chloride; B) 125 mM methyl alpha-D-mannopyranoside (α-MMP) in mobile phase A.

The conditions of separation: mobile phases gradient: 0–10.5 min 100% A, 11–20 min 100% B, 20–30 min 100% A, flow rate: 0.5 mL/min, column temperature: 25 °C, loop: 50 μL, detection: UV detection at 214.8 nm.

Collected proteins of non-bound and bound fractions were consequently subjected to dialysis against distilled water using dialysis cassettes with 3.5 kDa cut-off (Thermo Fisher Scientific, IL, USA), and lyophilized.

**SDS-PAGE**

After re-dissolving, the lyophilized fractions were subjected to SDS-PAGE electrophoresis performed according to the Laemmli procedure (1970). The separation of protein mixtures was carried out using gradient (4–20%) gel (Bio-Rad) gels under denaturing conditions. Visualization was performed by Coomassie Brilliant blue G-250.

**In-gel digestion**

Selected (glyco)protein bands were excised and enzymatically in-gel digested by trypsin or chymotrypsin according to Jensen et al. (1999) digestion protocol. Obtained peptides were purified by ZipTip C18 prior to MS analysis. A desalted solution of peptides was analyzed by MALDI-TOF MS.

**MALDI-TOF/TOF analysis**

An AB Sciex TOF/TOF 5800 System (AB Sciex, Framingham, MA) equipped with a 1 kHz Nd:YAG laser was used for this work. All experiments were performed in a positive reflectron mode. The samples (0.4 μL) were applied on the MALDI target by a dry-droplet method. Alpha-cyano-4-hydroxycinnamic acid [10 mg/mL 0.1% trifluoroacetic acid/acetoniitrite (1:1, v/v)] was used as a MALDI matrix for the analysis of peptides obtained by enzymatic digestion.

**Protein identification and database search**

Acquired mass spectra were processed using TOF/TOF Series Explorer version 4.1.0 (AB Sciex) and data were submitted to the MASCOT database searching. The NCBI-nr database was used for the search with taxonomy restriction to “other green plants”. Maximum tolerance for peptide masses was 100 ppm and fragment error was set as 0.3 Da. Additional parameters used: enzyme trypsin or chymotrypsin; allowed missed cleavages: up to 2, fixed modification: carbamidomethyl, variable modification: oxidation of methionine; peptide charge: +1; monoisotopic masses; instrument MALDI TOF/TOF.
Results

Description the purification of glycoproteins

Our attention was paid on analysis of water-soluble proteins and thus deionized water was selected as the extraction solvent. First, a small fraction was taken from each crude extract and was subjected to 1D-gel electrophoresis for quick screening of the protein profile. As expected, SDS gels showed that all examined cereals contained large number of proteins, mainly in the mass range up to 120 kDa. Visual inspection of gel revealed significant quantitative as well as qualitative differences at protein patterns of barley and wheat. On the other hand, only quantitative differences between durum and spelt were observed (Fig. 2). Since we focus on glycosylated proteins, which are usually in minority, the subsequent simplification of obtained complex protein mixture was required. Lectin affinity chromatography (AC) was used for enrichment of cereal glycoproteins. This work partially took up the previous study of our team (Laštovičková et al. 2011) where manually filled columns with ConA have been used for the purification of plant glycoproteins. To improve the efficiency of the affinity enrichment which is crucial in analysis of low abundant glycoproteins, we decided to perform the purification on a high capacity monolithic ConA HPLC column. Affinity HPLC were performed under experimental conditions optimized before (Benkovska et al. 2013) and briefly described in Materials and Methods. First, glycosylated proteins were bound to lectin stationary phase and non-bound proteins were washed away. After eleven minutes, bound glycoproteins were released using eluent containing alpha-D-mannopyranoside. Since one separation run took about 30 min and it was automated, the enrichment step was not time consuming. HPLC column provided high reproducibility, sensitivity, efficiency and fast performance. As the result of AC analysis, non-bound and bound fractions were obtained for each cereal samples. As an example, ConA AC separation profile of proteins isolated from durum is shown in Fig. 1. Bound fractions of all three cereals represent only minor part of total

![Figure 1. ConA HPLC chromatogram of durum proteins. The affinity purified glycoprotein was released by 125 mM methyl alpha-MMP and detected spectrophotometrically at 214.8 nm](image-url)
protein content. Levels of glycoproteins in individual cereals were comparable. Glycoproteins in ConA bound fractions comprised 5.53% of total content of protein for barley, 5.99% for durum and 4.54% for spelt, respectively. The particular fractions eluted from lectin column were dialyzed against distilled water, lyophilized and subsequently subjected to 1D gel electrophoresis with gradient gels under denaturing conditions. 1D SDS gel (Fig. 2) confirmed that the majority of cereal high-abundant proteins remained in non-bound AC fractions. Several (glyco)protein areas were found in each cereal fraction after ConA separation; most importantly bands at molecular masses of 70–55 kDa, 45–30 kDa and strong bands in low molecular weight region of 25–15 kDa. The presence of glycosylation of ConA bound proteins was indicated by the characteristic smearing bands caused by the heterogeneity of present glycan structures (different glycoforms attached to the same protein chain) (Kuster et al. 2001).

**Identification of glycoproteins**

The bands from chosen protein areas of estimated 70–15 kDa were consecutively in-gel digested by trypsin or chymotrypsin and subjected to MALDI-TOF MS analyses. Database identification of MS/MS data revealed origin of proteins for majority of gel bands.
### Table 1. Summary of proteins bound by ConA identified in barley flour

<table>
<thead>
<tr>
<th>Indication of gel spots</th>
<th>Accession number</th>
<th>Protein name</th>
<th>Molecular mass (Da)</th>
<th>Position of N-glycosylation triplet N-X-S/T</th>
<th>Function of identified protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>gi</td>
<td>326505038</td>
<td>Predicted protein</td>
<td>46 795</td>
<td>No potential glycosylation site</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>804656</td>
<td>Beta-glucosidase</td>
<td>57 694</td>
<td>86; 356; 423</td>
</tr>
<tr>
<td>B2</td>
<td>gi</td>
<td>326489434</td>
<td>Predicted protein</td>
<td>44 663</td>
<td>247; 245; 359; 422</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>326521432</td>
<td>Predicted protein</td>
<td>48 333</td>
<td>45; 187; 267</td>
</tr>
<tr>
<td>B3</td>
<td>gi</td>
<td>326498119</td>
<td>Predicted protein</td>
<td>33 991</td>
<td>85; 151</td>
</tr>
<tr>
<td>B4</td>
<td>gi</td>
<td>326513840</td>
<td>Predicted protein</td>
<td>55 847</td>
<td>228; 371; 405</td>
</tr>
<tr>
<td>B5</td>
<td>gi</td>
<td>2506771</td>
<td>Alpha-amylase inhibitor BMAI-1</td>
<td>16 376</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>123958</td>
<td>Alpha-amylase/trypsin inhibitor CM16</td>
<td>16 399</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>585290</td>
<td>Alpha-amylase/trypsin inhibitor CMb</td>
<td>17 199</td>
<td>124</td>
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</table>

### Table 2. Summary of proteins bound by ConA identified in wheat durum flour

<table>
<thead>
<tr>
<th>Indication of gel spots</th>
<th>Accession number</th>
<th>Protein name</th>
<th>Molecular mass (Da)</th>
<th>Position of N-glycosylation triplet N-X-S/T</th>
<th>Function of identified protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>gi</td>
<td>443481</td>
<td>Serine carboxypeptidase</td>
<td>28 889</td>
<td>116; 127</td>
</tr>
<tr>
<td>D2</td>
<td>gi</td>
<td>326498119</td>
<td>Predicted protein</td>
<td>33 991</td>
<td>85; 151</td>
</tr>
<tr>
<td>D3</td>
<td>gi</td>
<td>123963</td>
<td>0.19 dimeric alpha-amylase inhibitor</td>
<td>13 899</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>114215854</td>
<td>Dimeric alpha-amylase inhibitor</td>
<td>13 948</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>123957</td>
<td>Alpha-amylase/trypsin inhibitor CM3</td>
<td>18 893</td>
<td>No potential glycosylation site</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>123955</td>
<td>Alpha-amylase/trypsin inhibitor CM1</td>
<td>16 077</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>474323981</td>
<td>Globulin-1 S allele</td>
<td>55 586</td>
<td>349</td>
</tr>
</tbody>
</table>
Nevertheless, some protein bands, visible in the SDS gels, remained unidentified. We suppose that this might be the major reason for failed identification – protein is extensively modified. The troubles in interpreting of mass spectra of modified proteins probably come either from the fact that a significant number of peptides have poor fragmentation and/or from the fact that conventional database search algorithms miss modified peptides. The results of successful identifications based on MS/MS data are given in Tables 1–3. MS proteomic analysis provided information about amino acid sequence of separated proteins. Moreover, since N-glycosylation requires specific triplet of amino acids (N-X-T/S, where X can be any amino acid residue except P), MASCOT identification also provided information about location of potential site of N-glycosylation. As shown in Tables 1–3, not all proteins identified in the ConA retained fraction contained possible glycosylation site. The reason can be in retention of non-specifically bound proteins as the result of commonly occurring protein–protein interactions (Balonova et al. 2010). The second explanation may be some alterations in amino acid sequence of identified proteins which have not yet been listed in databases.

Some of the identified proteins are still named predicted proteins, especially in the grain sample. This means that this area of minor glycoproteins is still little studied. Several identified proteins are also known to act as sensitizing agents according to database. The N-linked glycans of plant and insect glycoproteins are the most abundant environ-

<table>
<thead>
<tr>
<th>Indication of gel spots</th>
<th>Accession number</th>
<th>Protein name</th>
<th>Molecular mass (Da)</th>
<th>Position of N-glycosylation triplet N-X-S/T</th>
<th>Function of identified protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>gi</td>
<td>113786</td>
<td>Beta amylase</td>
<td>59 894</td>
<td>249; 338; 402</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>393450</td>
<td>Beta amylase</td>
<td>57 008</td>
<td>123; 249; 338</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>32400764</td>
<td>Beta amylase</td>
<td>31 100</td>
<td>65; 154</td>
</tr>
<tr>
<td>S2</td>
<td>gi</td>
<td>326498119</td>
<td>Predicted protein</td>
<td>33 991</td>
<td>85; 151</td>
</tr>
<tr>
<td>S3</td>
<td>gi</td>
<td>123975</td>
<td>Endogenous alpha-amylase/subtilisin inhibitor</td>
<td>19 849</td>
<td>No potential glycosylation site</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>585290</td>
<td>Alpha-amylase/trypsin inhibitor CMb</td>
<td>17 199</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>123958</td>
<td>Alpha-amylase/trypsin inhibitor CM16</td>
<td>16 399</td>
<td>124</td>
</tr>
<tr>
<td>S4</td>
<td>gi</td>
<td>114215786</td>
<td>Dimeric alpha-amylase inhibitor</td>
<td>13 833</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>gi</td>
<td>66841026</td>
<td>Alpha-amylase inhibitor 0.19</td>
<td>13 340</td>
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</tr>
</tbody>
</table>
mental immune determinants. In barley, most of the allergenic proteins characterized so far belong to the α-amylase/trypsin inhibitor family. Both β(1-2) xylose and α(1-3) fucose have been found in glycosylated inhibitors BMAI-1 and CMb that were identified in our study in ConA captured fraction of barley grain (in case of BMAI-1) as well as spelt. BMAI-1 belongs to the family of plant proteins that inhibit alpha-amylases and proteinases from pests and pathogens and could be involved in insect defense mechanisms. The basic information about the identified proteins will be described in the next section. Similarly, summary of peptides obtained by MS measurement is presented in Table S1*.

Discussion

Identified glycoproteins were present in cereals as a mixture of isoforms differing only in several amino acids (e.g. beta amylase in spelt – see Table 3). They could be sorted according to different attributes (based on protein family and molecular function) into several groups. First group was formed by proteins called in databases as “predicted proteins” (see Tables 1–3). Associations, that are responsible for sequencing of new genomes, publish the information about the predicted genes and their translated products (= proteins) in public databases (e.g. NCBI Non-Redundant Protein Database (NCBInr)). Thus, existence of proteins called in databases only as “predicted proteins” has not been strictly proven but it is indicated on the basis of expression data (cDNA(s), RT-PCR or Northern blots). Our discovery of these predicted proteins in barley, durum and spelt indicates that cereal minor glycoproteins have been still poorly explored. Several identified proteins are still named – predicted proteins, especially in the grain sample. This implies that this area of minor glycoproteins is still little studied. The amino acid sequence corresponding to predicted protein (gi|326498119) is shown in Fig. 3a. Our experiments confirmed this protein in barley, durum and also in spelt flour.

Proteins belonging to the alpha-amylase/trypsin inhibitor family represented the second group of identified proteins (Tables 1–3). Our especial attention was focused on the identification of these low-molecular-weight proteins in ConA bound fraction because group of wheat and barley flour allergens with molecular masses about 10–30 kDa has been previously described (Perrocheau et al. 2005; Matsuo et al. 2010). Most of these proteins have been investigated with non-proteomic analytical methods. Protein BMAI-1 (barley monomeric alpha-amylase inhibitor) is endosperm glycoprotein representing a major allergen in baker’s asthma disease, which was found to be active against insect alpha-amylase (Sanchez-Monge et al. 1992; Laštovičková et al. 2011). The amino acid sequence of BMAI-1 inhibitor, which was confirmed in barley grain, is shown in Fig. 3b. It contains a single n-glycosylation site at asparagines at position 125. CM16, CMb (identified in barley and spelt) and CM3 and CM1 (discovered in durum) belong to “CM-proteins”. Wheat α-amylase/subtilisin inhibitor (WASI; detected in spelt) inactivates alpha-amylase from animal and insect origin and it is also able to inhibit subtilisin (Bønsager et al. 2005). CM proteins as well as WASI are connected with IgE wheat allergy (Sotkovsky et al. 2011).

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

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The third group of proteins comprised proteins with different functions (β-glucosidase (barley), globulin-1 S allele and serine carboxypeptidase (wheat/durum) or β-amylase (spelt). Generally, β-glucosidases are present in prokaryotes as well as in eukaryotes. Plant β-glucosidases participate in regulating the biological activity of plant phytohormones by releasing active forms from inactive hormone-glucoside conjugates (Leah et al. 1995). Globulin-1 S allele is a protein with unknown function. Nevertheless a related protein from maize (Zea mays) was characterized as seed storage protein with potential glycosylation site (www.uniprot.org/uniprot/P15590). A serine carboxypeptidase hydrolyzes a peptide bond at most three amino acid residues from the C-terminus of a polypeptide chain (www.ebi.ac.uk/QuickGO/GTerm?id=GO:0004185). A presence of this enzyme in both barley grain and malt has been already described by our team (Laštovičková et al. 2011). Serine carboxypeptidase was also identify in wheat durum flour (Table 2, spot D1). Generally, these enzymes are ubiquitous in higher organisms. Serine carboxypeptidases are usually glycoproteins with subunit molecular weights of 40,000–75,000 Da. The whole sequence of wheat serine carboxypeptidase with accession number gi|443481 is shown in Fig. 3c. It contains two potential N-glycosylation sites at position 116, 127. Allergic activity of serine carboxypeptidase from wheat and maize was explored by Weichel et al. (2006), when they confirmed the connection of serine carboxypeptidase with baker’s asthma. β-Amylases catalyze the hydrolysis of α-1,4 glycosidic bond, releasing maltose units from the non-reducing end of polysaccharides. Barley.

Figure 3. Amino acid sequence of “a” predicted protein (gi|326498119), “b” alpha-amylase inhibitor BMAI-1 (gi|2506771), “c” serine carboxypeptidase (gi|443481). The trios of amino acids marked in bold print represent the potential N-glycosylation sites.
β-amylase is used for production of glucose syrups rich in maltose obtained by degradation of starch from wheat, barley and maize grains. These syrups are used in confectionery. Barley β-amylase is classified as a potentially allergenic protein (Weichel et al. 2006). As was described in paragraphs above, proven protein allergens, especially those with low-molecular-weight, were discovered in all investigated cereals.

Our results showed that the combination of lectin HPLC-GE-MALDI-TOF MS/MS is useful method for global screening of glycoproteins in cereal materials. The universality of this method is supported by a fact that a choice of lectin enable to influence the character of enriched cereal glycoprotein fraction according to the purposes of investigation. Presented proteomic based protocol improved the knowledge about cereal (glyco)proteins with affinity to ConA lectin. Twenty-four N-glycoprotein candidates from barley, durum and spelt have been successfully identified. Several of those belong to confirmed allergens. The applied method was also capable of revealing proteins which have not been described as glycoproteins so far. Especially a group of detected “predicted proteins” seems to be promising subject to more detailed study.

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References


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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary Table S1. Summary of peptides obtained by MS measurement