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REGULAR ARTICLE

STUDY OF WHEAT PROTEIN DEGRADATION DURING GERMINATION

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ABSTRACT

Glutens, the storage proteins of cereals, play significant role in technological and nutraceutical quality of cereal grains. Whereas the high content of glutens allows making better structure of dough, consummation of foods with high gluten content can cause digestive problems. In our work, we studied ability of wheat proteases to degrade proteins, especially glutens. Wheat grains were germinated for up to seven days at three different temperatures 15, 20, 30 °C and pH 3.0, 4.0, 5.5, 7.0, 8.0. Proteins were fractionated into salt-soluble albumins and globulins, alcohol-soluble gliadins and base-soluble glutenins. In these fractions, protein content and their composition were analyzed by Bradford method and SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The results showed that remarkable degradation of glutens started after three days and the lowest concentration was measured at the seventh day of germination of wheat grain at temperature 20 °C, pH 5.5.

Keywords: wheat, proteins, germination, hydrolysis, proteases

INTRODUCTION

Wheat is an important part of the daily diet of millions of people but wheat products consumed in various forms are also responsible for food allergies **(Larré *et al.*, 2011)**. Coeliac disease, also known as coeliac sprue and gluten sensitive enteropathy, is a one of the most frequent food intolerances worldwide **(Wieser and Koehler, 2008)**. It may be defined as an inflammatory disease of the small intestine triggered by the storage (gluten) proteins not only from wheat but also from rye, barley and possibly, oats **(Gessendorfer *et al.*, 2010)**.

Wheat proteins are classically divided into two main groups: the salt soluble fraction mainly composed of albumins and globulins, and the gluten fraction made up of gliadins and glutenins. The gluten fraction was reported to be responsible for coeliac disease and later on food allergy **(Larré *et al.*, 2011)**. Albumins and globulins are mostly biologically-active, performing catalytic and regulating functions. They are constitutional proteins with enzymatic activity. Wheat's gliadins and glutenins, also called prolamins, function primarily as storage proteins **(Waga, 2004)**. Gliadins are single polypeptide chains, while glutenins are multi-chained structures of polypeptides (subunits) linked by disulfide bonds. The overall structure of gliadins consists of a central domain containing repetitive amino acid sequences rich in proline and glutamine, and two terminal non-repetitive domains which are more hydrophobic and contain most of the ionisable amino acid, although the latter are present only in low levels **(Thewissen *et al.*, 2010)**.

The role of the proline- and glutamine-rich storage proteins of cereals is to supply the embryo with nitrogen and amino acids during the first period of seedling development. Therefore, it is likely that endogenous cereal proteases synthesized during germination would be capable of extensively hydrolyzing these proteins **(Hartmann *et al.*, 2006)**. These peptidases have distinct advantages when compared to bacterial and fungal peptidases and are promising candidates for the detoxification of gluten-containing foods and possibly for oral therapy of coeliac disease patients. They are composed of endopeptidases and exopeptidases and have unique specificities optimized by nature for the fragmentation of gluten protein and peptides **(Gessendorfer *et al.*, 2011)**.

The aim of this work was studied degradation of both protein groups, biological -active (albumins and globulins) and storage (glutenins and gliadins) proteins, by wheat´s proteases during germination of wheat grains at various conditions.

MATERIAL AND METHODS

Material

Winter wheat (*Triticum aestivum* L.) grain of the cultivar "Šarlota" (bread quality) cultivated in year 2010 was obtained from Selekt – Bučany a. s. (Slovak Republic).

Germination

Wheat grains were immersed in distilled water for 12 hours at 20 °C. After the water decanted and washing with distilled water, the wheat grains were germinated on a piece of watered cotton in Petri dishes at three different temperatures (12, 20, 30 °C) and at five different pH (3.0, 4.0, 5.5, 7.0 and 8.0) in the dark during 7 days. Samples collected at daily intervals were dried at 60 °C for 48 hours. After drying, the sprout and rootlets were eliminated and the remaining seeds were homogenized to particles < 1 mm.

Extraction proteins

Wheat germinated grains (0.25 g) were suspended in 2.5 mL 10 % NaCl, stirred for 45 minutes and centrifuged at 4000 g for 10 minutes. The extraction was repeated three times. The combined supernatants contained albumins and globulins were analysed along. The pellet remaining after the NaCl extraction was extracted with 2.5 mL 70 % (v/v) ethanol to separate gliadins. The extraction was carried out three times for 45 minutes and the obtained mixture was centrifuged at 4000 g for 10 minutes. The pellet remaining after the extraction of gliadins was treated with 2.5 mL 0.2 % NaOH, stirred for 45 minutes and centrifuged. Alkaline extraction was repeated three times. The combined supernatants were used to obtain the glutenin fraction **(Karamać *et al.*, 2007)**. The gliadin and glutenin fractions extracted from germinated grains present together gluten fraction.

Protein quantifications

The Bradford assay relies on the binding of the dye is a rapid Coomassie Brilliant Blue G-250 to protein. Towards 10 µL of protein fraction was added 300 µL of Bradford reagent. After 5 minutes, absorbance at 600 nm was determined. Bradford reagent was prepared by

dissolving 100 mg Coomassie Brilliant Blue G-250 (Merck) in 50 mL of 95 % ethanol. The solution was then mixed with 100 mL of 85 % (w/v) phosphoric acid and made up to 1 L with distilled water **(Kruger, 2002)**. Concentration of proteins was expressed as mg proteins per gram of dry mass.

SDS-PAGE electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), to determine the molecular weight of proteins in prepared fractions, was performed with a 12 % polyacrylamide containing 0.1 % sodium dodecyl sulfate as described by **Laemmli (1970)**. The buffer solution for the separating gel was Tris-HCl 1.5 M (pH 8.8). 15 μL of protein extracts from different extraction conditions (NaCl or ethanol) with different pH (3.0 and 5.5) were loaded onto the gel and electrophoresed with Tris-glycine buffer (pH 8.3). Electrophoresis was performed at 180 V for 35 minutes. The protein bands were visualized by Commassie Brilliant Blue G-250 (Merck) and compared with standard molecular weight markers 2 – 212 kDa (BioLabs).

Statistical analysis

All samples were analysed in triplicate. The results were processed using the statistical software Microsoft Excel 2010.

RESULTS AND DISCUSSION

By Bradford method, the concentration of albumins and globulins has been increased during germinated wheat grains from the first to sixth day of germination (12 °C – 15.9 mg g^{-1}, 20 °C – 18.6 mg g^{-1}, 30 °C – 15.3 mg g^{-1}). Concentration of these proteins have been markedly decreased during seven day of germination (12.6 mg g^{-1}) at 20 °C and only slightly at 12 °C and 30 °C (14.4 mg g^{-1} and 13.5 mg g^{-1}, respectively) (Fig 1).

Using of buffer with different pH the concentration albumins and globulins fell below after the fourth day of germination with exception pH 7.0 (Fig 2a). **Mikola and Jones (2000)** found, that hydrolysis by cysteine peptidases pass off faster at low value pH and very low is degradation at pH 6.2. This finding agree with our achieved results because lowest

degradation of these proteins was determined at pH 4.0, 7.0, 8.0 and the best hydrolysis came through at pH 3.0 (15 mg g^{-1}).

In process germination of wheat grain the concentration albumins and globulins have been increased because the process germination of grain is accompanied by increasing of hydrolytic enzyme activities.

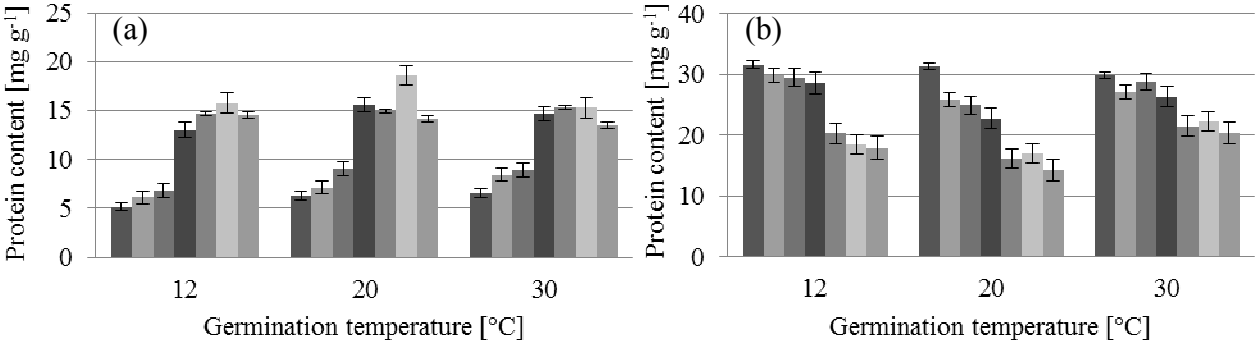

Figure 1 Contents of albumins and globulins (a) and glutens (b) in germinated wheat at pH 5.5 and different temperatures during seven days. ■ – 1. day, ■ – 2. day, ■ – 3. day, ■ – 4. day, ■ – 5. day, ■ – 6. day and ■ – 7. day

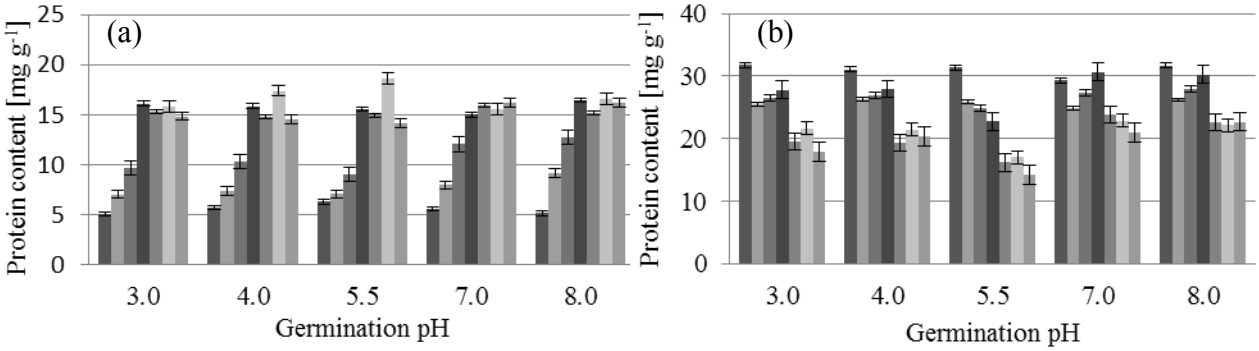

Figure 2 Contents of albumins and globulins (a) and glutens (b) in germinated wheat at 20 °C and different pH values during seven days. ■ – 1. day, ■ – 2. day, ■ – 3. day, ■ – 4. day, ■ – 5. day, ■ – 6. day and ■ – 7. day

Bottari *et al.* (1996) found that since the cysteine proteases represents the bulk of the activity of the germinating wheat grains and is able to cleave the gliadin to small peptides. It appears to play a key role in the initial degradation of the storage proteins present in the starch endosperm. By Bradford method, the lowest content of gluten was found in germinated grains

during seven days (14 mg g^{-1}) at 20 °C, pH 5.5 (Fig 1b). Our results to confirm of ascertainment of **Bigiarini *et al.* (1995)** that effect activity of cysteine peptidase after the four day of germination and also opinion of **Hartmann *et al.* (2006)** that at low temperature has been in progress of gluten hydrolysis faster.

According to **Bottari *et al.* (1996)** the optimal pH for gluten hydrolysis is acid pH which agrees with our findings. The low concentration of glutens was determined (17.9 mg g^{-1}) at the seventh day of germination at pH 3.0 (Fig 2b). Our experiments result that during germination, the ratio between content of biological-active (albumins and globulins) and storage (glutens) proteins change. Whereas the content of biological-active proteins increased, storage proteins were degraded. This finding point out that germination of wheat grain is significant tool for decreasing of gluten in wheat grains using for production of gluten – free or gluten – low foods.

SDS-PAGE

SDS – gel electrophoresis of proteins extracted from wheat germinated at pH 3.0 and 5.5 (20 °C), and from ungerminated wheat, using a 10 % NaCl (albumins and globulins) and 70 % ethanol (gliadins) is shown in Fig 3. SDS-PAGE has been widely used for separation proteins from all cereals. Albumins are water-soluble proteins with a relatively low molecular weight of about 17 to 28 kDa and globulins salt-soluble proteins with a relatively low molecular weight of about 25 to 300 kDa **(Urminská and Bašista, 2005)**.

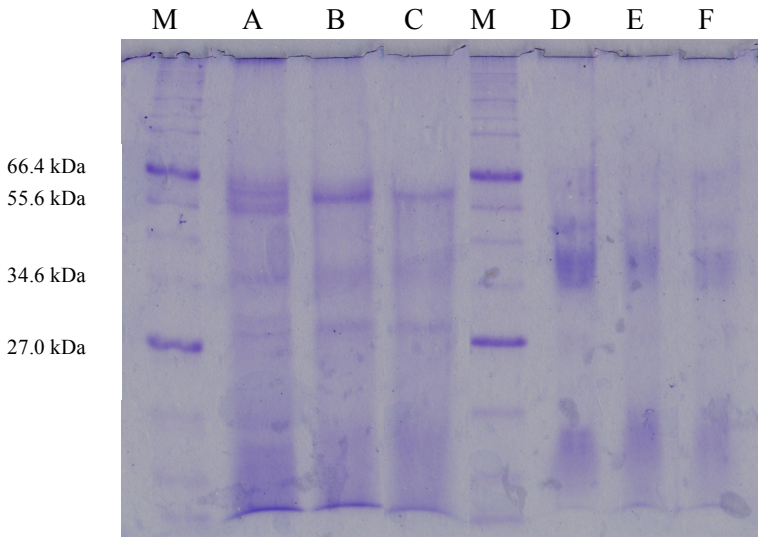

Figure 3 Protein profile of *Triticum aestivum* L. **Lane M:** molecular weight standard, **Lane A:** albumins and globulins of ungerminated wheat, **Lane B:** albumins and globulins of germinated wheat at pH 3.0 and 20 °C, **Lane C:** albumins and globulins of germinated wheat at pH 5.5 and 20 °C, **Lane D:** gliadins of ungerminated wheat, **Lane E:** gliadins of germinated wheat at pH 3.0 and 20 °C, **Lane F:** gliadins of germinated wheat at pH 5.5 and 20 °C

The protein fraction, albumins and globulins of ungerminated wheat (Lane A), were hydrolyzed by germinated wheat proteases in the area with molecular weight 66.4 – 55.6 kDa. The primary allergic proteins are prolamins of wheat grain with a relatively low molecular weight of about 30 to 80 kDa **(van Eckert *et al.*, 2010)**. In our experiments, we found that germinated wheat proteases hydrolyzed gliadins with molecular weight 42.7 – 34.6 kDa at pH 5.5 (Lane F) better than at pH 3.0 (Lane E) and temperature 20 °C, same as albumins and globulins. **Bottari *et al.* (1996)** determined that cysteine protease represents the bulk of the activity of the germinating wheat seeds and is able to cleave the gliadin to small peptides. It appears to play a key role in the initial degradation of the storage proteins present in the starchy endosperm.

CONCLUSION

The experiments have demonstrated that germinated wheat proteases able to degrade glutens. However, the hydrolysis of the glutens started after the three day of germination of wheat grains and the lowest concentration of proteins were achieved at the seventh day of

germination at 20 °C, pH 5.5. This determination was confirmed by electrophoresis. Gliadins were hydrolyzed mainly in the area with molecular weight 42.7 – 34.6 kDa.

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