JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

2-DE PROTEOME MAPS OF AMARANTH AND BUCKWHEAT SEEDS

Zdenka Gálová, Eva Pálenčárová, Milan Chňapek, Želmira Balažová*

Address(es): prof. RNDr. Zdenka Gálová, CSc.,
Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak Univesity of Agriculture, Trieda A. Hlinku 2, SK-949 76 Nitra, Slovak Republic.

*Corresponding author: Zdenka.Galova@uniag.sk

ARTICLE INFO

Received 17. 10. 2013
Revised 19. 11. 2013
Accepted 16. 12. 2013
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

Our work was focus on amaranth (*Amaranthus* sp.) cv. Plaisman and buckwheat (*Fagopyrum* Mill.), cv. Pyra proteome, which was analysed by 2-D electrophoresis. We found similarity between the chemical properties proteins of pseudocereals amaranth and buckwheat. Image analysis showed a higher number of spots on 2-DE map of buckwheat in comparison to amaranth. Some similarities were in protein spots at approximately 21,000 Da, pI 7 and strip of protein spots in range of pI 8-10, 21,000 Da. The buckwheat 2-DE map shows spots of protein with higher intensity in the region ranging from 30-45,000 Da, pI 5-6 as well as highly abundant protein spots from visible at 36-40,000 Da, pI 8-9. Protein maps showed that the pseudocereals do not content storage proteins, which indicates that they are suitable as a replacement for cereals for people with celiac disease.

Keywords: Amaranth, buckwheat, 2-DE protein maps, celiac disease

INTRODUCTION

Coeliac disease (CD) is a gluten-sensitive enteropathy characterized primarily by gastrointestinal symptoms and malabsorption. The triggering agent in CD is believed to be the prolamine constituent gliadin of wheat and prolamines from rye and barley. Coeliac disease (CD) constitutes permanent intolerance to dietary wheat proteins, especially gluten. The protein intolerance of CD is mediated by the enhanced gastrointestinal mucosal immune system, which is activated (**Trier, 1991**)
Gluten has positive effect on technological properties of wheat and bread-making, but on the other hand it is trigger of coeliac disease in genetically susceptible individuals. It is known that CD is caused by the alcohol-soluble storage proteins (prolamins) of wheat (gliadins), rye (secalins), and barley (hordeins). There is still disagreement about oat prolamins (avenins), whereas prolamins from other cereals are not harmful to coeliac patients. Investigations of the relationship between prolamin structure and toxicity have only been done with gliadin from common (bread) wheat. *In vivo* and *in vitro* studies indicated that all major gliadin types (α-, γ-, and ω-gliadins) produce toxic effects **(Wieser, 2008)**. Gliadins can be heated or digested into peptides without loss of toxicity. Thus, tertiary structures of the gliadins are not important, but small peptides arising from gliadins by gastrointestinal enzymes are responsible for the toxic effects **(Wieser, 2001)**. Some cereals (rice, maize and others) and pseudocereals (amaranth, buckwheat, quinoa), rich in proteins, do not contain gluten, which is the main cause of celiac disease.
The objectives of our work was to prepare and compare 2-DE protein maps of amaranth and buckwheat from the point of view detection of coeliac active proteins.

MATERIAL AND METHODS

Plant material

Seeds of Amaranth (*Amaranthus* sp.) cv. Plaisman and seeds of buckwheat (*Fagopyrum* Mill.), cv. Pyra were used for analyses. Seeds from the Gene Bank of the Research Institute of Plant Production, Piešťany (Slovak Republic) were obtained and by CU Mill, (Lionhill Company a.s.) were milled to a homogenous flour.

Methods

Two - dimensional gel electrophoresis (2-DE)

Samples preparation

Proteins were extracted from the flour by adding 1 ml of buffer [250 µl DTT (28 mg/ml), 12.5 µl IPG buffer (carrier ampholytes) and 237.5 µl ultra-pure water to 2 ml IPG rehydration buffer (7 M urea, 2 M thiourea, 2 % CHAPS)] to 50 mg of flour. The samples were then wheel-mixed for 1 h, RT and then centrifuged 3 min, 9, 000 x g, RT. The protein content of the supernatant (SN) was estimated by Coomassie Plus protein assay (Thermo Scientific, Pierce, UK) (based on the Bradford assay) and samples were stored at -20 ºC until use. The protein content of the oat extract was insufficient, so the Compact-Able™ Protein Assay Preparation Reagent Set (Thermo Scientific, Pierce, UK) was used to precipitate the protein, which was then re-suspended in the extraction buffer as described previously.

1st Dimension - Isoelectric focussing (IEF): Immobilised pH gradient (IpG) strips (GE Healthcare, Amersham UK), 7 cm, pH 3-11 NL and pH 6-11, were used for the first dimension. Strips were hydrated O/N at 20°C 125 µl rehydration buffer [7 M urea; 2 M thiourea, 2 % w/v CHAPS; 0.5 M DTT; relevant pH range IPG buffer; 0.001 % w/v bromophenol blue] containing ~ 40 µg protein of sample. Focussing was performed at 20 ºC, current 50 µA *per* strip (300V 30 min 0.2 kVh; 1000 V 30 min, 0.3 kVh; 5000 V, 1 h 20 min, 4.0 kVh; 5000 V, 25 min, 2.0 kVh). Focussed IPG strips were stored at -80 ºC until required.

2nd Dimension - SDS PAGE: Focussed IPG strips were equilibrated in tris-acetate equilibration buffer [0.122 M tris-acetate containing 0.5 % w/v SDS; 6 M urea; 3 % w/v glycerol; 52 mM DTT; 0.01% w/v Bromophenol blue]. After 30 min strips were derivatised in the dark with 0.14 M iodoacetamide in equilibration buffer for a further 30 min. Strips were then transferred to 1 mm, 4-12 % Bis-Tris Zoom™ gels for the second dimension. Gels were run at 200 V and 100 W per gel for 35 min using 1 x MES SDS Running Buffer. Gels were fixed O/N in 40 % v/v methanol containing 10 % w/v TCA before staining with SYPRO Ruby Stain (Invitrogen, UK) in the dark O/N. After de-staining O/N with 10 % v/v methanol and 6 % TCA, gels were imaged using a high-resolution molecular imager (PHAROS FX™ Plus, Bio Rad, UK). Imaged gels were returned to de-stain solution and stored in the dark at 4 °C until required.

RESULTS AND DISCUSSION

It is known that alcohol soluble prolamins are predominate in cereals. Globulins are predominant in legumes and other dicotyledones. Recent findings suggest that Western diets based on highly palatable foods are likely to be much less satiating than more ethnic foods or those typical of less developed countries. In particular, some alternative crops (e.g. buckwheat, oat, barley, spelt, rye, quinoa, amaranth) seem to be of great nutritional interest and to represent important recipes for healthier and typical regional foods **(Aubrecht and Biacs, 2001; Gorinstein et al., 2002)**. Therefore, in the last decade, the use of pseudocereals was increased not only in special diets for people allergic to cereals, but also in healthy diets. Comparative protein studies of cereals and pseudocereals are important, especially in cases of cereal protein allergy when pseudocereal substitution is unavoidable **Gorinstein et al., (2005)**.

At the present time, a very prospective group of crops that can be consumed as part of a gluten-free diet for CD appear to be so called pseudocereals. They include buckwheat, amaranth, quinoa etc. Unfavourable fractions and proteins (e.g. gluten) are not present in these botanically different species compared to cereal grasses **(Petr et al., 2003).**

Cereals and, to a lesser degree, pseudocereals are an important part of the human diet and provide key nutrients including proteins, antioxidants and minerals. Therefore, the investigation of allergen-free pseudocereals has been intensified and the use of them recommended. Based on its rich protein and amino acid compositions, amaranth could be a nutritive substitute for cereals **(Gorinstein et al., 2002; Wieser and Koehler, 2008)** for CD sufferers. Pseudocereals contain relatively high amounts of dietary fiber, which improves lipid metabolism and takes part in prevention of LDL (Low-density lipoprotein) cholesterol oxidation. After processing, these plants can be used as flours or flakes or in biscuits and breakfast foods **(Krkošková and Mrázová, 2005**).

The aim of our study was to evaluate the electrophoretic profiles of storage proteins of amaranth seeds and buckwheat seeds, which were obtained by two-dimensional electrophoresis (2-DE). Two–dimensional gel electrophoresis has frequently been used to characterize the diversity of protein components. The first dimensional involves isoelectric focusing, in which proteins are fractionated across a specific pH range using commercially available pH gradient strips. The second-dimension fractionation resolves the proteins on the basis of molecular mass, using sodium dodecyl sulfate polyakrylamide gel electrophoresis (SDS-PAGE) **(Skylas et al., 2000).**

The 2-DE protein maps in figure 1 represents the proteins of mature amaranth seed flour. First, 2-DE - gels with pH 3-11 were run, that were followed by 2-DE - gels with pH 3-11. Upon initial observation, amaranth has many proteins focussed between pH 3-11, 25, 000-200, 000 Da (figure 1) with areas of abundant proteins at: 14, 000-31,000 Da, pI 8-9 (Figure 1 v), 36, 000 Da, pI 4-6 (figure 1 vii), 55, 000-66, 000 Da , pI 6-7 (figure 1 viii); this could be 11S globulin which has a theoretical pI 6.53, M_rs 55,064 Da, and belongs to the 11S seed storage protein family. Fewer proteins were observed for the pH 6-11 gel, especially in the HMW weight region of the gel where storage proteins (figure 1i, 1ii) were observed for the cereals. This is due to the fact that the pseudocereals are a botanically different species compared to cereal grasses, and even if they are rich in protein, unfavourable fractions are not present or are only available in small amounts.

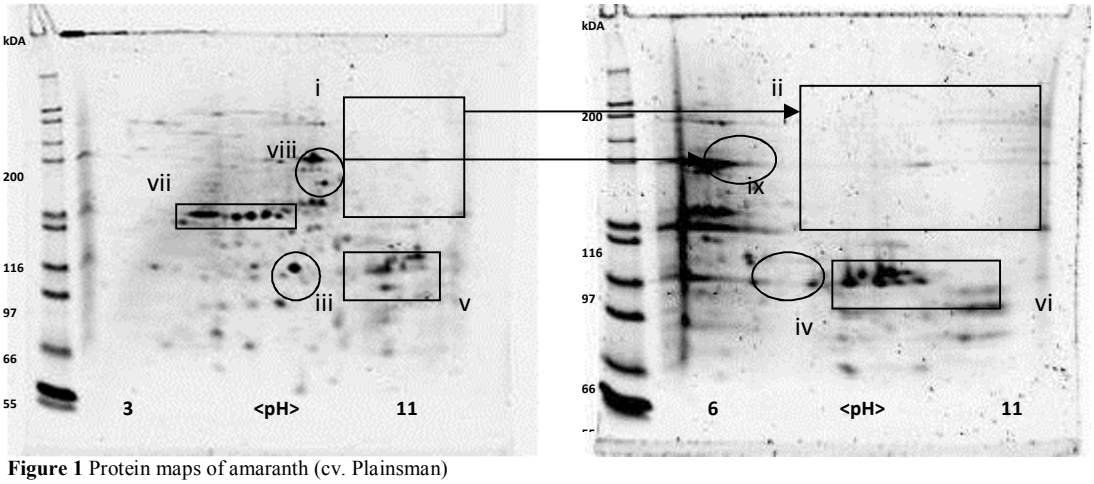

Figure 1 Protein maps of amaranth (cv. Plainsman)

The 2-DE protein maps in figure 2 represents the proteins of mature buckwheat seed flour. Similar to amaranth, buckwheat has many proteins focussed in the acidic region and fewer in the basic region; of which these proteins appear to be highly abundant. Also, in comparison to Amaranth, the lack of storage proteins in the HMW basic regions of the gels (figure 2i, 2ii) was noticeable (Mrs from 36-200,000 Da, pI 7-11). Image analysis showed a higher number of spots on 2-DE map of buckwheat when compared to amaranth (figure 1). Also, the buckwheat 2-DE map shows spots of protein with higher intensity in the region ranging from 30-45,000 Da, pI 5-6 (figure 2vii) as well as highly abundant protein spots from visible at 36-40,000 Da, pI 8-9 (figure 2viii). We can see some similarities with buckwheat cv. Pyra. For example, protein spots at approximately 21,000 Da, pI 7 (figures 1iii, 1iv and figures 2iii, 2iv), a strip of protein spots in range of pI 8-10, 21,000 Da (figures 1v, 1vi and figures 2v, 2vi).

We can notice big differences especially between protein composition of cereals and pseudocereals **(Pálenčárová, Gálová, 2010**). On the pseudocereal proteins maps we miss protein pattern in area with molecular mass ranging from 40 to 200,000 Da and pI ranging from 9-11, which are visible on cereal proteins maps and corresponded to gluten protein these are supposed triggers of celiac disease. This showed differences between species that also are related to differences in functional properties.

There is not enough information about 2-DE of pseudocereals available in the literature so it was difficult to assign any likely protein identifications to the gels. For the moment we has not realized mass spectrometry experiments for exact identification protein spots, as well as using sera from coeliac patients to identify problem proteins, but it belongs to our future plans.

Gorinstein et al., (2005) analysed the relationship between dicotyledons (amaranth, quinoa, fagopyrum, soybean) and monocots (sorghum and rice), based on protein analyses and their use as substitution of each other and found similarities between these plants, which could make them a substitute of each other as well as for cereals. They reported that combination and substitution of cereals by pseudocereals lead to nutritional foods and can prevent allergy. Food components may be promoters of positive metabolic mechanisms; **Gupta (2004)** says that a combination of cereals, pseudocereals and soybean provides protein-rich ingredients resulting in higher nutritive value.

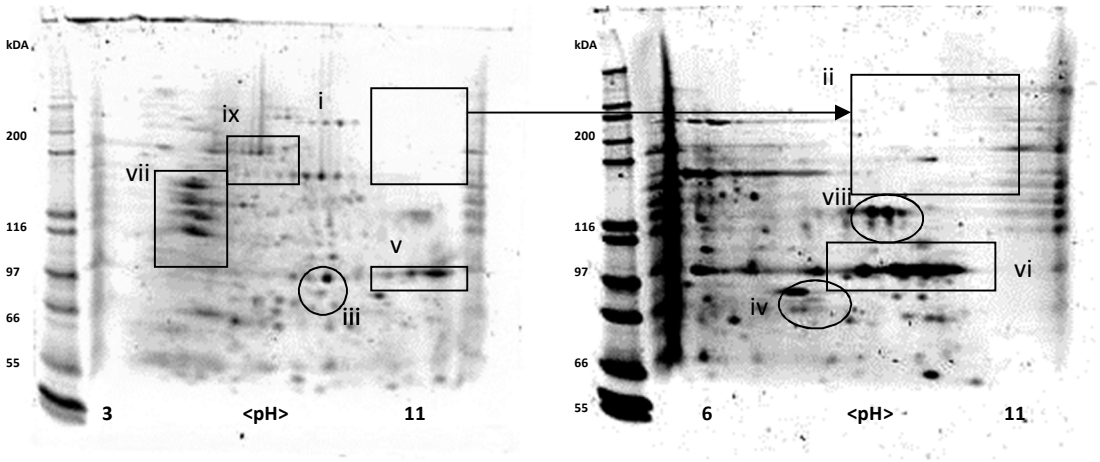

Figure 2 Protein maps of buckwheat (cv. Pyra)

CONCLUSION

Our work deals with comparison of amaranth and buckwheat 2-DE proteins maps with aim to find out differences between then. We determined similarity between the chemical properties proteins of pseudocereals amaranth and buckwheat, where most of the extracted proteins have pI-values ranging from 5 to 10 and the molecular masses ranging from 14 to 55,000 Da. Analysed pseudocereals did not show the presence of celiac active proteins.

Acknowledgments: This work was co-funded by European Community under project no 26220220180: Building Research Centre „AgroBioTech" (50 %) and VEGA project No.1/0513/13 (50 %).

REFERENCES

AUBRECHT, E., BIACS, P. A. 2001. Characterisation of buckwheat grain proteins and its product. In *Acta Alimentaria*, vol. 30, 2001, no 1, p. 71-80.

GORINSTEIN, S., DRZEWIECKI, J., DELGADO-LICON, E., PAWELZIK, E., AYALA, A. L. M., MEDINA, O. J., HARUENKIT, R., TRAKHTENBERG, S. 2005. Relationship between dicotyledone-amaranth, quinoa, fagopyrum, soybean and monocots- sorghum and rice based on protein analyses and their use as substitution of each other. In *Journal of European Food Research and Technology,* vol. 221, 2005, p. 69-77.

GORINSTEIN, S., PAWELZIK, E., DELGADO-LICON, E., HARUENKIT, R., WEISZ, M., TRAKHTENBERG, S. 2002. Characterization of pseudocereals and cereals proteins by protein and amino acid analyses. In *Journal of the Science of Food and Agriculture,* vol. 82, 2002, p. 886-891.

GUPTA, H. O. 2004. Improving the nutritional quality of maize after suplementation with processed soybean. In *Journal of Food Science and Technology*, vol. 41, p. 167-170.

KRKOŠKOVÁ, B., MRÁZOVÁ, Z. 2005. Prophylactic components of buckwheat. In *Food Research International*, vol. 38, 2005, p. 561-568.

PALENČÁROVÁ, E., GÁLOVÁ, Z. 2010. Detection of celiac active proteins by electrophoretic and immunochemical methods [CD-ROM]. In *Potravinárstvo*. Nitra : SPU v Nitre, vol. 4, 2010, p. 485-490. ISSN 1338-0230.

Trier JS. Coeliac sprue. N Engl J Med 1991;325:1709–19.

PETR, J., MICHALÍK, I., TLASKALOVÁ, H., CAPOUCHOVÁ, I., FAMĚRA, O., URMINSKÁ, D., TUČKOVÁ, L., KNOBLOCHOVÁ, H. 2003. Extention of the spectra of plant products for the diet in coeliac disease. In *Czech Journal of Food Scientia*, roč. 21, 2003, č. 2, s. 59-70.

SKYLAS, D. J., MACKINTOSH, J. A., CORDWELL, S. J., BASSEAL, D. J., WALSH, B. J., HARRY, J., BLUMENTHAL, C., COPELAND, L., WRIGLEY, C. W., RATHMELL, W. 2000. Proteome approach to the characterisation of protein composition in the developing and mature wheat-grain endosperm. In *Journal of Cereal Science,* vol. 32, 2000, p.169-188.

WIESER, H., KOEHLER, P. 2008. The Biochemical Basis of Celiac Disease. In *Cereal Chemistry*, vol. 85, 2008, no. 1, p. 1-13.

WIESER, H. 2001. Comparative investigations of gluten proteins from different wheat species. III. N-terminal amino acid sequences of α-gliadins potentially toxic for coeliac patients. In *European Food Research and Technology*, vol. 213, 2001, p. 183-186.

WIESER, H., KOEHLER, P. 2008. The Biochemical Basis of Celiac Disease. In *Cereal Chemistry*, vol. 85, 2008, no. 1, p. 1-13.