Proteomic Analysis of Wheat α/A- and β-Gliadins

Marta DZIUBA[1], Dorota NAŁĘCZ[1], Iwona SZERSZUNOWICZ[1] and Jacek WAGA[2]

[1]Department of Food Biochemistry, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; [2]Cereals Department, Plant Breeding and Acclimatization Institute, Kraków, Poland

Abstract

Dziuba M., Nałęcz D., Szerszunowicz I., Waga J. (2014): **Proteomic analysis of wheat α/A- and β-gliadins**. Czech J. Food Sci., **32**: 437–442.

Gliadins from the Polish common winter wheat cultivar Sukces were analysed by analytical and preparative A-PAGE combined with 2-DE method. The main aim of this study was to identify the highest possible number of α/A- and β-gliadin fractions. Gliadins from the wheat cv. Sukces were separated by 2-DE into 82 spots. Preparative A-PAGE combined with the 2-DE method supported the identification of the analysed gliadin fractions. 12 spots were identified as typical α/A-gliadins out of 40 and 7 as typical β-gliadins out of 15 separated by 2-DE.

Keywords: wheat gliadins; 2-DE, A-PAGE

Gliadin proteins are present in most of wheat food products and are important in food industry and human nutrition all over the world. As gluten components they influence the baking quality of wheat flour. On the other hand, they are alergenic proteins causing IgE mediated alergies. Gliadins are monomeric wheat storage proteins which play a key role in celiac disease pathogenesis. Their structure and amino acid composition constitute the chemical basis of gliadin-induced toxicity. Similarly to other prolamin group proteins, gliadins are rich in glutamine (around 40% content) and proline (around 14% content) (Tatham & Shewry 1985; Dewar *et al.* 2004). They are one of the most complex and polymorphic protein groups in the plant kingdom. Gliadins of a single wheat genotype are composed of hundreds of polypeptides controlled by six complex loci on chromosomes belonging to the 1st and 6th homology groups (*1A*, *1B*, *1D*, *6A*, *6B*, and *6D*) of the hexaploid wheat genome (*Triticum aestivum* L.) (Payne 1987; Waga *et al.* 2011). Particular loci comprise from several to several hundred closely linked genes which are inherited as clusters. Non-lethal mutations during wheat evolution differentiated the clustered genes into numerous allelic variants

grouped into multiple gene families. Based on their mobility in acidic polyacrylamide gel electrophoresis (A-PAGE), gliadins are divided into α-, β-, γ-, and ω-classes (Tatham & Shewry 1985). Polypeptides belonging to one class are usually controlled by genes located on two or more chromosomes.

In both analytical and preparative applications, A-PAGE is the basic method of gliadin identification. Based on A-PAGE results, the gliadin complex extracted from one wheat genotype may be separated into dozens of protein fractions (Waga & Zientarski 2007; Waga *et al.* 2011). Proteomic analysis, developed in the last two decades, greatly contributed to the identification of gliadins and research into gliadin activity in celiac disease. The most important tool in proteomics is two dimensional electrophoresis (2-DE) which combines isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Carbonaro 2004; Görg *et al.* 2004; Dziuba *et al.* 2007, 2009; Nałęcz *et al.* 2009; Rabilloud *et al.* 2009). The 2-DE method supports effective separation of hundreds to thousands proteins per analysis. Despite the advancements in proteomic and genomic technologies, the identification and analysis of gliadins continues to pose a problem

due to their complex structure and extensive polymorphism (Mamone *et al.* 2005; Ferranti *et al.* 2007; Pompa *et al.* 2013). The results of A-PAGE have demonstrated that cv. Sukces, a Polish winter wheat cultivar, contains a combination of protein bands characteristic of α/A-gliadins.

The objective of this study was to isolate, identify and characterise the gliadin fractions of the wheat cv. Sukces which is one of the most important wheat cultivars in Poland of high bread-making quality. The classic, basic method of gliadin identification, A-PAGE, was combined with two dimentional electrophoresis (2-DE) to increase the effectiveness of the analysis.

MATERIAL AND METHODS

Material. Kernel samples of Polish winter wheat cv. Sukces harvested in eastern Poland were analysed in this study.

Chemical reagents for A-PAGE (acrylamide, *N,N'*-methylenebisacrylamide, aluminum lactate and lactic acid), and staining reagents (Coomassie Brilliant Blue R-250 and G-250) were supplied by Sigma-Aldrich (Munich, Germany). The reagents for 2-DE, including ethanol, methanol, acetic acid, and 85% *o*-phosphoric acid were purchased from Polish Chemical Reagents (Gliwice, Poland). Roti®-Blue staining solution (5× concentrated Colloidal Coomassie G-250) was supplied by Carl Roth (Karlsruhe, Germany). The other reagents used were purchased from Sigma-Aldrich (Munich, Germany).

Gliadins extraction. Gliadins for A-PAGE were extracted stepwise from flour samples prepared from wheat cv. Sukces. Albumins and globulins were discarded using 0.15M NaCl for 2 hours. After centrifugation (15 000 *g*, 20°C, 5 min), 70% ethanol was added to the pellets (1 : 10 w/v). The samples were gently shaken overnight and repeatedly centrifuged. The obtained ethanol extracts were condensed with sucrose in lactate aluminum buffer, pH 3.1, and electrophoresed (Bernardin *et al.* 1967).

The gliadin complex for 2-DE was extracted according to the method proposed by Weiss *et al.* (1993) and Simpson (2004). Ethanol supernatants containing gliadins were pooled, lyophilised, and stored at –80°C.

Protein concentration was measured in gliadin extracts as described by Bradford (1976) using the Bio-Rad protein assay (Bio-Rad, cat. No. 500-0006) and bovine serum albumin (Bio-Rad, cat. No. 500-0007) as the protein standard.

Isolation of α*/A- and β-gliadins by ultracentrifugation*. 200 ml of 0.01M acetic acid was added to 50 g of wheat flour (cv. Sukces), the mixture was left for 2 h and centrifuged (10 000 g, 20°C, 30 min). The obtained supernatant was ultracentrifuged (137 000 *g*, 4°C, 2 h) in the Beckman apparatus. The supernatant produced by ultracentrifugation was discarded, and 5 ml of distilled water was added to the pellet for 24 hours. The suspension was centrifuged (10 000 *g*, 20°C, 20 min), and the resulting supernatant was mixed with acetate buffer, pH 5.1. Ultracentrifugation was repeated on the following day (130 000 *g*, 4°C, 2 h). The pellet containing α/A-gliadins and β-gliadins was lyophilised and stored at 4°C (Bernardin *et al.* 1967).

Purification of α*/A- and β-gliadins by preparative A-PAGE*. Proteins isolated by ultracentrifugation were further purified by preparative A-PAGE in aluminum lactate buffer, pH 3.1. Each separation was performed in the Model 491 PrepCell (BioRad, Raleigh, USA). A preparative column (diameter 37 mm, height 100 mm) was filled with polyacrylamide gel with total monomer (T) concentration of 8% (w/v) and crosslinker (C) concentration of 3.61% (w/v). The strongly exothermic polymerisation reaction required intensive column cooling during the gel preparation. For the separation, 6 ml of the mixture containing the gliadin extract and saturated sucrose solution in lactate aluminum buffer was applied on the gel surface. Each separations was performed at constant voltage (U = 500 V) and current (I = 50 mA). Sixty aliquots of 7 ml containing gliadin fractions were collected beginning 5 h after the start of electrophoresis using pH 3.1 aluminum lactate buffer as eluent. The separation was completed 12 h after the onset of fraction collection (Bernardin *et al.* 1967; Waga & Zientarski 2007; Waga *et al.* 2011).

2-D electrophoresis of gliadin proteins and their α*/A- and* β*-fractions*. Two dimensional electrophoresis (2-DE) was used to analyse both extracted complex of gliadin proteins and α/A- and symbol β-gliadin fractions purified by preparative A-PAGE (Görg 2004; Dziuba *et al.* 2009).

Image analysis. Destained gels were processed in the Image Master 2-D Platinum 6.0 computer program (GE Healthcare, Upsala, Sweden) with the use of the Melanie algorithm (Sanchez *et al.* 1995). Isoelectric points, molecular weights, and spot volumes of all separated proteins were calculated. Each sample was separated by 2-DE in three replications, and all of them were analysed digitally.

Statistical analysis. The average value and standard error of the mean (SEM) were calculated for

individual isoelectric points, molecular weights, and spot volumes. The statistical significance of differences between isoelectric point (pI), molecular weight (MW), and spot volume of the selected, characteristic total gliadin and α/A-gliadin spots was estimated by Student's *t*-test. Statistical analysis was performed using Statistica 10.0 PL software (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Gliadins from the wheat cv. Sukces were separated by 2-DE into 82 spots (pI 3.2–9.6; MW 24.3–68.4 kDa). Only 20 of 82 spots were identified as typical gliadins with characteristic protein isoelectric points (6.75–8.24) and molecular weights (31.41–40.51 kDa) (Figure 1). The region of the 2-DE polypeptide map which was most likely to contain α/A- and β-gliadins (pI 6–9 and MW 21–40 kDa) was determined based on the results of our previous study where gliadins were characterised by RP-HPLC (Dziuba *et al.* 2007) and 2-DE (Konopka *et al.* 2007).
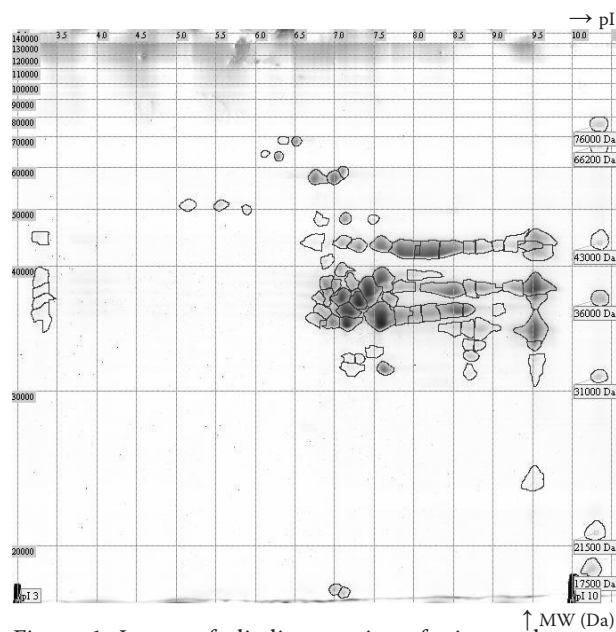

Figure 1. Image of gliadin proteins of winter wheat cv. Sukces separated by 2-DE. Masses of molecular markers are shown on the right. Numbers of 20 characteristic gliadin spots are shown in the 2-DE image

Table 1. Proteomic characteristics of selected α/A- (Figure 2b) and β-gliadins (Figure 2c) from wheat cv. Sukces, isolated by preparative A-PAGE and separated by 2-DE

No.	Isoelectric point[1] (pI)	Molecular weight[1] (MW, kDa)	Spot volume[1] (%)[2]
α/A-gliadins			
1	6.80 ± 0.01	34.20 ± 0.10	2.03 ± 0.44
2	6.88 ± 0.01	39.28 ± 0.67	1.45 ± 0.08
3*	6.98 ± 0.01*	35.60 ± 0.11*	1.72 ± 0.14*
4	7.14 ± 0.01	34.03 ± 0.11	5.38 ± 0.19
5	7.15 ± 0.01	34.83 ± 0.12	1.84 ± 0.12
6	7.17 ± 0.01	31.32 ± 0.11	1.02 ± 0.06
7*	7.27 ± 0.01*	35.56 ± 0.14*	5.07 ± 0.58*
8	7.54 ± 0.01	34.64 ± 0.10	1.80 ± 0.10
9	7.51 ± 0.04	39.45 ± 0.18	1.11 ± 0.03
10	7.63 ± 0.03	34.17 ± 0.21	7.96 ± 0.49
10	7.64 ± 0.03	34.83 ± 0.18	4.77 ± 0.26
12	7.66 ± 0.01	31.26 ± 0.12	1.95 ± 0.07
13	7.74 ± 0.03	35.52 ± 0.23	2.18 ± 0.03
14	8.35 ± 0.15	34.91 ± 0.22	6.03 ± 0.02
β-gliadins			
1*	6.93 ± 0.01*	36.06 ± 0.03*	1.44 ± 0.08*
2	7.23 ± 0.01	35.04 ± 0.03	1.03 ± 0.08
3*	7.23 ± 0.01*	35.94 ± 0.04*	3.78 ± 0.90*
4	7.33 ± 0.03	38.49 ± 0.06	1.84 ± 0.36
5	7.65 ± 0.01	36.46 ± 0.33	1.91 ± 0.28
6.	7.79 ± 0.01	37.92 ± 0.09	7.22 ± 1.39
7.	9.85 ± 0.03	33.35 ± 0.02	2.51 ± 0.08

[1]mean value of three repetitions ± SEM (standard error of the mean); [2]calculated based on 2-DE separated proteins and expressed in % of the total spot volume of all separated proteins; *spots present in images of both on α/A- and β-gliadins

Gliadins from winter wheat cv. Sukces were separated by A-PAGE into 24 electrophoretic bands corresponding to individual gliadin fractions (Figure 2a). Fractions A, B, and C belong to the group of ω-1.2 gliadins, fractions D–G were classified as ω-5 gliadins, fractions H–N as γ-gliadins, fractions O–R as β-gliadins, and fractions S–X as α-gliadins. The electrophoretic pattern of α-gliadins is characteristic of A-gliadins which are highly aggregable proteins controlled by genes located on short arm of chromosome *6A* in *Gli A2* locus. In the analysed cultivar, they are represented by electrophoretic bands T, U, and V.

The gliadin protein complex was separated into several α-, β-, γ-, and ω-gliadin fractions using preparative A-PAGE. The fractions containing individual purified α- and β-gliadins were further analysed by 2-DE (Figures 2b and 2c).

Despite different electrophoretic mobilities, α- and β-gliadins have similar protein structures and are often classified into a single group of α/β-gliadins. Similarly to other gluten proteins, they contain mainly glutamine and proline residues and both repetitive and specific sequence regions. a/β-Gliadins contain around 100 amino acids in repetitive N-terminal
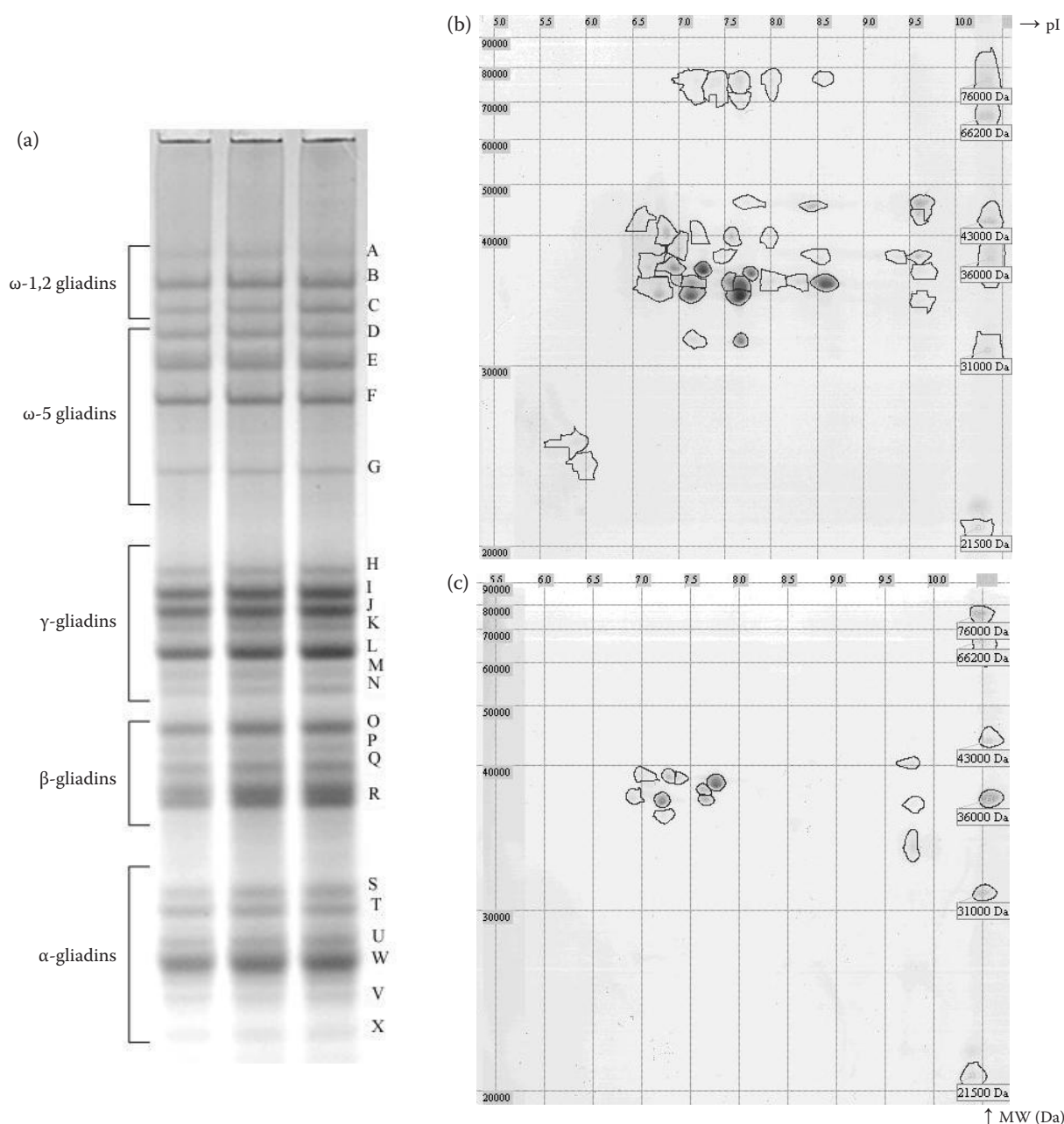

Figure 2. Gel image fragments of separated gliadin proteins from wheat cv. Sukces: (a) analytical A-PAGE of gliadin fractions (marked by letters A–X), (b) 2-DE of α/A-gliadins, and (c) 2-DE of β-gliadins

Table 2. Matched spots for all gliadins (Figure 1) and α/A-gliadins (Figure 2b) from wheat cv. Sukces

No.	Gliadins[1]		α/A-Gliadins[2]	
	isoelectric point[3] (pI)	molecular weight[3] (MW, kDa)	isoelectric point[3] (pI)	molecular weight[3] (MW, kDa)
1	6.75 ± 0.03[a]	34.42 ± 0.13[a]	6.80 ± 0.01[a]	34.20 ± 0.10[a]
2	6.89 ± 0.03[a]	40.51 ± 0.03[a]	6.88 ± 0.01[a]	39.28 ± 0.67[a]
3*	7.12* ± 0.06[a]	36.22* ± 0.29[a]	6.98* ± 0.01[a]	35.60* ± 0.11[a]
4	7.10 ± 0.03[a]	34.24 ± 0.28[a]	7.14 ± 0.01[a]	34.03 ± 0.11[a]
5	7.17 ± 0.06[a]	34.92 ± 0.19[a]	7.15 ± 0.01[a]	34.83 ± 0.12[a]
6	7.22 ± 0.03[a]	31.55 ± 0.03[a]	7.17 ± 0.01[a]	31.32 ± 0.10[a]
7*	7.27* ± 0.03[a]	35.57* ± 0.19[a]	7.27* ± 0.01[a]	35.56* ± 0.14[a]
8	7.62 ± 0.09[a]	34.78 ± 0.47[a]	7.61 ± 0.01[a]	34.58 ± 0.14[a]
9	7.56 ± 0.02[a]	38.09 ± 0.26[a]	7.51 ± 0.04[a]	39.45 ± 0.18[a]
10	7.65 ± 0.08[a]	31.41 ± 0.17[a]	7.66 ± 0.01[a]	31.26 ± 0.12[a]
11	7.70 ± 0.03[a]	36.03 ± 0.03[a]	7.74 ± 0.03[a]	35.52 ± 0.23[a]
12	8.24 ± 0.17[a]	34.92 ± 0.17[a]	8.35 ± 0.15[a]	34.91 ± 0.22[a]

[1]gliadins separated by 2-DE; [2]α/A-gliadins isolated by preparative A-PAGE and separated by 2-DE; [3]mean value of three repetitions ± SEM; *spots present in the image of β-gliadins; [a]no significant differences were observed between pI and MW values of individual gliadin spots at $P ≤ 0.05$ (Student's t-test)

sequences and around 200 amino acids in specific C-terminal sequences of polypeptides. N-terminal sequences of α/β-gliadins contain 5 amino acid residues, while C-terminal sequences are not clearly separated from the specific region. α/β-Gliadins are sulfur-rich proteins containing six cysteines in a specific region. Cysteines in α/β-polypeptides form intramolecular disulfide (SS) bonds which influence protein folding and produce protein structures which are more compact than in other gluten protein groups. The most typical repetitive sequences in the repetitive region are heptapeptide PQPQPFP and pentapeptide PQQPY (Ferranti *et al.* 2007).

Forty protein spots located between pI 5.91 and 9.61 and MW between 24.4 kDa and 75.9 kDa were identified in 2-DE image of the selected α/A-gliadins (Figure 2b). The comparison of 2-DE electropherograms of isolated α/A-gliadins (Figure 2b) and the gliadin complex (Figure 1) of wheat cv. Sukces revealed 14 spots with statistically significant identity. They were located in the pI range of 6.8–8.4 and the MW range of 31.26–39.45 kDa. The volumes of the individual spots were calculated as the percentage of total spot volume (Table 1).

The β-gliadin fraction (Figure 2c) isolated by preparative A-PAGE was divided into 15 protein spots. Seven of the 15 identified spots were classified as typical β-gliadins (Table 1). They were located in the pI range of 6.93–9.85 and in the MW range of 33.35–38.49 kDa.

Two α/A-gliadin spots (pI 6.98, MW 35.60 kDa and pI 7.27, MW 35.56 kDa) were also present in the group of isolated β-gliadins (Figures 2b–c, Table 1). Twelve protein spots of gliadins from wheat cv. Sukces were identified as α/A-gliadins (Table 2) based on the results of the analysis performed.

The identification and analysis of gliadins continue to pose a problem due to their complex structure and extensive polymorphism. Individual gliadins differ in isoelectric points, molecular weights, and amino acid sequences which further complicates the analytical efforts (Waga *et al.* 2011). In this study, gliadins from wheat cv. Sukces were separated by 2-DE into 82 spots. Only 20 gliadin spots were identified as typical gliadins with characteristic isoelectric points and molecular weights. Preparative A-PAGE combined with the 2-DE method supported the identification of the analysed gliadin fractions. 12 spots were identified as typical α/A-gliadins out of 40 and 7 as typical β-gliadins out of 15 separated by 2-DE.

References

Bernardin J.E., Kasarda D.D., Mecham D.K. (1967): Preparation and characterization of α-gliadin. Journal of Biological Chemistry, **242**: 445–450.

Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72**: 248–254.

Carbonaro M. (2004): Proteomics: present and future in food quality evaluation. Trends in Food Science & Technology, **15**: 209–216.

Dewar D., Pereira S.P., Ciclitira P.J. (2004): The pathogenesis of coeliac disease. International Journal of Biochemistry & Cell Biology, **36**: 17–24.

Dziuba J., Minkiewicz P., Nałęcz D., Dziuba M., Szerszunowicz I. (2009): Reproducibility of two-dimensional electrophoresis gel images of pea (*Pisum sativum* L.) seed proteins evaluated using scatter plots – a short report. Polish Journal of Food and Nutrition Sciences, **59**: 143–146.

Dziuba J., Nałęcz D., Minkiewicz P., Hanasiewicz A. (2007): Application of ultraviolet spectroscopy to discriminate wheat α/β and γ-gliadins separated with high-performance liquid chromatography – a short report. Polish Journal of Food and Nutrition Sciences, **57**: 191–193.

Ferranti P., Mamone G., Picariello G., Addeo F. (2007): Mass spectrometry analysis of gliadins in celiac disease. Journal of Mass Spectrometry, **42**: 1531–1548.

Görg A. (2004): 2-D Electrophoresis. Principles and Methods. Handbooks from GE Healthcare. GE Healthcare Bio-Sciences AB, Uppsala: 73–75, 133–136.

Görg A., Weiss W., Dunn M.J. (2004): Current two-dimensional electrophoresis technology for proteomics. Proteomics, **4**: 3665–3685.

Konopka I., Fornal Ł., Dziuba M., Czaplicki S., Nałęcz D. (2007): Composition of proteins in wheat grain streams obtained by sieve classification. Journal of the Science of Food and Agriculture, **87**: 2198–2206.

Mamone G., Addeo F., Chianese L., Di Luccia A., De Martino A., Nappo A., Formisano A., De Vivo P., Ferranti P. (2005): Characterization of wheat gliadin proteins by combined two-dimensional gel electrophoresis and tandem mass spectrometry. Proteomics, **5**: 2859–2865.

Nałęcz D., Dziuba J., Minkiewicz P., Dziuba M., Szerszunowicz I. (2009): Identification of oat (*Avena sativa*) and buckwheat (*Fagopyrum esculentum*) proteins and their prolamin fractions using two-dimensional polyacrylamide gel electrophoresis. European Food Research and Technology, **230**: 71–78.

Payne P.I. (1987): Genetics of wheat storage proteins and the affect of allelic variation on bread-making quality. Annual Review of Plant Physiology, **38**: 141–152.

Pompa M., Giuliani M.M., Palermo C., Agriesti F., Centonze D., Flagell Z. (2013): Comperative analysis of gluten proteins in three durum wheat cultivars by a proteomic approach. Journal of Agricultural and Food Chemistry, **61**: 2606–2617.

Rabilloud T., Vaezzadeh A.R., Potier N., Lelong C., Leize-Wagner E., Chevallet M. (2009): Power and limitations of electrophoretic separations in proteomics strategies. Mass Spectrometry Reviews, **28**: 816–843.

Sanchez J.C., Appel R.D., Golaz O., Pasquali C., Ravier F., Bairoch A., Denis F., Hochstrasser D.F. (1995): Inside SWISS-2D-PAGE database. Electrophoresis, **16**: 1131–1151.

Simpson R.J. (2004): Sequential extraction of wheat seed (*T. aestivum*) proteins. Purifying Proteins for Proteomics. A Laboratory Manual. Cold Spring Harbor Laboratory Inc., New York: 408–411.

Tatham A.S., Shewry P.R. (1985): The conformation of wheat gluten proteins. The secondary structure and thermal stabilities of the alpha-, beta-, gamma- and omega-gliadins. Journal of Cereal Sciences, **3**: 103–113.

Waga J., Zientarski J. (2007): Isolation and purification of individual gliadin proteins by preparative acid polyacrylamide gel electrophoresis (A-PAGE) for allergenic research. Polish Journal of Food and Nutrition Sciences, **57**: 91–96.

Waga J., Obtułowicz K., Zientarski J., Czarnobilska E., Skoczowski A. (2011): Purified wheat gliadin proteins as immunoglobin E binding factors in wheat mediated allergies. American Journal of Plant Sciences, **2**: 476–483.

Weiss W., Vogelmier C., Görg A. (1993): Electrophoretic characterization of wheat grain allergens from different cultivars in bakers' asthma. Electrophoresis, **14**: 805–816.

Corresponding author:

Dr inž. Marta Dziuba, Department of Food Biochemistry, University of Warmia and Mazury in Olsztyn, Pl. Cieszyński 1, 10-957 Olsztyn, Poland; E-mail: niklema@uwm.edu.pl