

IMMUNOREACTIVE PROPERTIES OF WHEAT CV. TONACJA STORAGE PROTEINS INFECTED WITH *FUSARIUM GRAMINEARUM* FUNGI

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The impact of *Fusarium graminearum* on the composition and immunoreactive properties of storage protein fractions of wheat has been evaluated. The study demonstrated that infection of non-germinated wheat grain with *Fusarium graminearum* does not change the immunoreactive properties of the peptides released. The electrophoretic characteristics of proteins did not show any changes in the quantitative nor qualitative composition of gliadins. Significant changes were observed in the composition of gliadin proteins isolated from germinating grain of wheat infected with *Fusarium graminearum*. The immunological characteristics of proteins demonstrated the presence of a polypeptide with a molecular weight ranging from 20 kDa to 24 kDa that reacted with antibodies obtained from blood of patients with gluten intolerance, which indicates that fusarium proteases may also release polypeptides potentially dangerous to health from storage proteins of wheat.

INTRODUCTION

The major pathogens colonizing cereals are fungi of the following genera: *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* [Placinta *et al.*, 1999]. Fungi of the genus *Fusarium* cause quantitative and qualitative losses in the crop. Ear blights result in embryo damage or a decreased mass of kernels, weakened germination force or infection with mycotoxins. Ear blight of wheat is induced by the following *Fusarium* species: *F. culmorum*, *F. graminearum*, *F. avenaceum* and *F. poae* [Prange *et al.*, 2005; Zhou *et al.*, 2005]. Infections with fusarium fungi are likely to evoke changes in the composition of saccharides, lipids and proteins of cereal kernels upon the activity of synthesized enzymes, *e.g.* amylases, chitinases, cellulases, glucanases, xylanases or proteases [Boyacioglu & Hettiarachchy, 1995; Pawelzik *et al.*, 1998].

By infecting cereals, *Fusarium* synthesizes proteases capable of decomposing storage proteins of kernels, thus modifying their composition and properties [Bechtel *et al.*, 1985]. Flour obtained from infected grain contains proteases which induce hydrolysis of endosperm proteins during fermentation of dough, thus contributing to deterioration of its technological properties and a reduced bread volume [Nightingale *et al.*, 1999].

Proteins are known to serve a variety of functions in food. A number of them demonstrate specific biological properties, often activated during digestive processes proceeding in the gastrointestinal tract. Peptides released from proteins by enteric proteolytic enzymes may exhibit regulatory properties similar to those displayed by hormones [Korhonen *et al.*, 1998] or allergenic properties [Tsuji *et al.*, 2001].

Allergenic proteins have been detected in grains of such cereals as wheat, barley, rye, oat as well as in maize, sorghum and buckwheat [Mills *et al.*, 2003]. One of recognized symptoms of allergenicity of cereal proteins is coeliac disease, asthma, dermatological changes, anaphylaxis evoked by physical exercise [Tsuji *et al.*, 2001].

Investigations into causative agents of the above-mentioned diseases demonstrated that they were induced by, among others, consumption of products containing wheat proteins [Rocher *et al.*, 1995]. Storage proteins of wheat grains initiate a chain of not completely recognized reactions, thus inducing activation of the immune system in genetically-predisposed persons [Gentile *et al.*, 2002].

Allergenic peptides are released as a result of hydrolysis of storage proteins of cereals by their native enzymes [Bleulkx *et al.*, 1997] and enzymes of bakery starter microflora [Rollán *et al.*, 2005]. The third source of proteolytic enzymes, capable of modifying kernel proteins, are fungi of *e.g.* the genus *Fusarium*.

Fungal proteases and released products of hydrolysis of kernel proteins of cereals infected with fungi may display toxic and allergenic activity and exert a negative effect on the quality of products obtained from them. The research was aimed at determining the impact of *Fusarium graminearum* on the composition and immunoreactive properties of storage protein fractions of wheat.

MATERIALS AND METHODS

Wheat grain samples

The experimental material were grain samples of winter wheat cv. Tonacja from the crop at the Experimental Sta-

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tion of the University of Warmia and Mazury (UWM) in Balcyny.

Fusarium strain

Use was made of a fungi strain *F. graminearum* obtained from the Chair of Plant Diagnostics and Pathophysiology UWM in Olsztyn.

Conditions of the activity of proteases of fungi from the genus *Fusarium* on wheat proteins during grain dormancy

PDA-agar culture medium was inoculated with fungi on Petri dishes. Sterile gauze with a diameter of *ca.* 10 cm was stretched over a plate so as not to touch the agar's surface. Wheat grain (2 g), previously sterilized with UV rays (2 h), was mounted on the gauze and incubated (4 days, 21°C) until the onset of sporulation of the *Fusarium* genus fungi and grain coverage with mould, but not allowing germination. The mould-grown grain was measured for the activity of endoproteolytic enzymes. Gliadins were extracted from all samples collected, then separated with electrophoretic techniques (CGE, SDS-PAGE) and analysed with the semi-dry immunoblotting method with the use of blood serum obtained from patients with symptoms of food allergy to gluten.

Conditions of the activity of proteases of fungi from the genus *Fusarium* on wheat proteins during germination of grain

The experiment was carried out according to a modified method of Pekkarinen *et al.* [2003]. Fungi of *Fusarium graminearum* were incubated for 120 h, at a temp. of 21°C on liquid PDA medium. The after-culture fluid was then centrifuged (8500×g, 15 min, 5°C), and the supernatant obtained (4 mL) was suspended in sterile redistilled water (30 mL) and thoroughly mixed. Wheat grains (56 g), previously sterilized with UV rays (2 h) and put into sterile bottles (100 mL, Schott), were poured into the prepared solution and thoroughly mixed.

After 21 h, after the grain had absorbed the whole fluid, they were distributed in equal amounts on sterile Petri dishes padded with sterile inserts made of filter paper (56 g/m², Filtrak, Poland) moistened with sterile redistilled water (1.3 mL). Dishes with *Fusarium* spores were incubated at a temperature of 21°C for 3 consecutive days.

A control sample was prepared by flooding grains with 34 mL of sterile redistilled water not containing *Fusarium graminearum* spores. Measurements were carried out in two replications.

Before and after incubation, the grains were measured for the activity of proteolytic enzymes. Gliadin was extracted, characterised with electrophoretic techniques (CGE, SDS-PAGE) and analysed with the semi-dry immunoblotting method with the use of blood serum obtained from patients with symptoms of food allergy to gluten.

Measurement of endoproteolytic activity

The activity of endoproteases was measured according to the method of Preston modified by Bleukx *et al.* [1997]. The substrate in the enzymatic reaction was azocasein (Sigma

11610). One unit of endoproteolytic activity (U_{EA}) was expressed as the change in sample absorbance at a wavelength of 440 nm over 1 h at 37°C and pH 5.5.

Extraction of gliadins

Extraction of gliadin fractions was carried out following the method of Weiss *et al.* [1993]. Wheat grains were ground in a grinder. The obtained flour (1 g) was suspended in a Tris-HCl buffer (4 mL, 0.05 mol/L, pH 6.8) and shaken (1 h, 5°C). The obtained extract was centrifuged (10,000×g, 30 min, 5°C), and the supernatant containing albumins and globulins was removed. Extraction with a Tris-buffer was repeated. The remaining precipitate was rinsed with de-ionized water (5 mL) and centrifuged (10,000×g, 30 min, 5°C). The precipitate was suspended in 2-propanol (4 mL, 750 mL/L) and extracted (2 h, 25°C). The sample was then centrifuged (10,000×g, 30 min, 5°C), and the supernatant obtained was collected. Extraction of prolamine fractions with 2-propanol (750 mL/L) was repeated two times. Supernatants containing gliadins were frozen.

Capillary gel electrophoresis (CGE) of proteins

The molecular weights of proteins were analysed with a capillary electrophoresis system by BioFocus 3000 (BioRad, USA), equipped with a UV-Vis detector. The preparation of samples and electrophoretic separations were carried out following the instructions of a kit for determination of molecular weights (BioRad, catalogue No. 148-4160, 148-4161). The quantitative and qualitative composition of proteins was analysed based on migration time and peak area sizes with the use of BioFocus Integrator software (BioRad, USA).

Polyacrylamide gel electrophoresis (SDS-PAGE) of proteins

The electrophoretic separation of proteins was carried out in a 12.5 g/L polyacrylamide gel using 40 g/L thickening gel (0.75 mm) in an electrophoretic apparatus Mini-PROTEAN 3 Cell (BioRad, USA). Samples of proteins (20 μL) were mixed with a sample's buffer (20 μL), thermally-denatured at 95°C for 5 min, and after cooling down were transferred into wells in 20 μL portions. The electrophoretic separation was carried out at ambient temperature at the following parameters: 90 V for 20 min and 130 V for *ca.* 120 min. The weight standard (Sigma M4038) were proteins with molecular weights ranging from 6.5 kDa to 205 kDa.

Immunoblotting of proteins

The electrophoretically-separated proteins were transferred from the polyacrylamide gel onto a nitrocellulose membrane, NC 2 (SERVA) in an MINITRANS apparatus for the so-called 'wet' electrotransfer (Kucharczyk TE, Poland). The electrotransfer was run in a Tris-glycine buffer with methanol (0.192 mol/L glycine; 0.025 mol/L Tris; 200 mL/L methanol, pH 8.3), applying a current with a strength of 25 mA, for 2 h, according to the methodology of Towbin *et al.* [1979].

Immunoblotting was carried out with the indirect method. Nitrocellulose membranes, containing electrophoretically-separated storage proteins of wheat (prolamines), were fixed in a TBS buffer (0.05 mol/L Tris-HCl buffer with the addi-

tion of 0.5 mol/L NaCl, pH 7.0), containing 2 mL/L Tween 20 (TBS-T) in order to block free sites binding protein. Next, the membranes were incubated (24 h, 4°C) in serum obtained from patients with symptoms of food allergy to gluten. After triple rinsing in a TBS-T solution, antigen-antibody complexes were visualized onto the membrane by fixing it in a solution of goat antibodies (IgG) specific to human immunoglobulin E, conjugated with horseradish peroxidase (Sigma A-9667) at a conjugate dilution of 1:2000. The incubation was run for 2 h at a room temperature. After the reaction of an enzyme with a substrate: 4-chloro-1-naphthol (Sigma C-6788) in the presence of H₂O₂, bands with a dark navy blue colour were observed to appear. The reaction was stopped by immersing the membranes in distilled water.

RESULTS AND DISCUSSION

While attacking a kernel, *Fusarium* synthesize a variety of enzymes that destroy its coats and facilitate penetration of the endosperm. First starch was hydrolyzed, then protein matrix [Jackowiak *et al.*, 2005].

Results of investigations carried out by different authors confirm changes in the quantitative and qualitative composition of storage proteins of kernels of wheat infected with *F. graminearum* [Bechtel *et al.*, 1985; Boyacioglu *et al.*, 1995; Jackowiak *et al.*, 2005]. Nightingale *et al.* [1999] showed that *Fusarium* endoproteases degrade proteins of cereal kernels to polypeptides with a lower molecular weight.

Effect of proteases of *Fusarium graminearum* fungi on wheat proteins during grain dormancy

The experiments have demonstrated small changes in the endoproteolytic activity in the cereal kernels examined. The endoproteolytic activity in grain of non-infected wheat and wheat infected with *Fusarium graminearum* accounted for 0.70 U_{EA}/mg protein and 0.82 U_{EA}/mg protein, respectively (Table 1). After the 96th h of incubation of non-infected and infected wheat grains, the activity of endoproteases increased 1.7- and 1.9-fold and reached 1.22 U_{EA}/mg protein and 1.58 U_{EA}/mg protein, respectively.

TABLE 1. Endoproteolytic activity in germinated and non-germinated wheat grains cv. Tonacja contaminated with *Fusarium graminearum*.

Sample	Incubation time (h)	Endoproteolytic activity (U _{EA} /mg protein)	
		\bar{x}	SD
Healthy non germinated wheat grains (control sample)	0	0.70	0.039
	96	1.58	0.017
Non germinated wheat grains contaminated with <i>F. graminearum</i>	0	0.82	0.050
	96	1.22	0.048
Healthy germinated wheat grains (control sample)	0	0.66	0.065
	96	2.50	0.021
Germinated wheat grains contaminated with <i>F. graminearum</i>	0	1.18	0.043
	96	2.85	0.040

U_{EA} – One unit of the endoproteolytic activity was expressed as a change in sample absorbance at a wavelength of 440 nm within 1 h at 37 °C and pH 5.5; \bar{x} – mean value; SD – standard deviation; n=6.

The low activity of endoproteases, probably caused by the presence of protease inhibitors, was confirmed by electrophoretic separations of prolamines of wheat infected with *Fusarium graminearum* carried out in this study. The CGE analysis of wheat prolamines did not demonstrate any changes in the molecular weights of proteins. Numbers of subfractions and values of molecular weights of proteins of wheat infected with *Fusarium* and non-infected wheat were comparable. Molecular weights measured ranged from 36 kDa to 76 kDa irrespective of the sample examined.

These experiments confirm the results obtained by Prange *et al.* [2005]. The *F. culmorum*, *F. cerealis*, *F. avenaceum* and *F. poae* fungi did not hydrolyse the storage proteins of wheat grains of Ambras and Rektor cultivars. Similar research by Pekkarinen *et al.* [2003] did not demonstrate any qualitative changes in storage proteins of barley after 33 and 46 days of infection with *F. culmorum*.

The SDS-PAGE electrophoretic separations of gliadins, as well as immunoblotting carried out in the study did not show any differences in the immunoreactivity of separated subfractions of gliadins isolated from grains of wheat infected with *Fusarium graminearum* and from non-infected grains

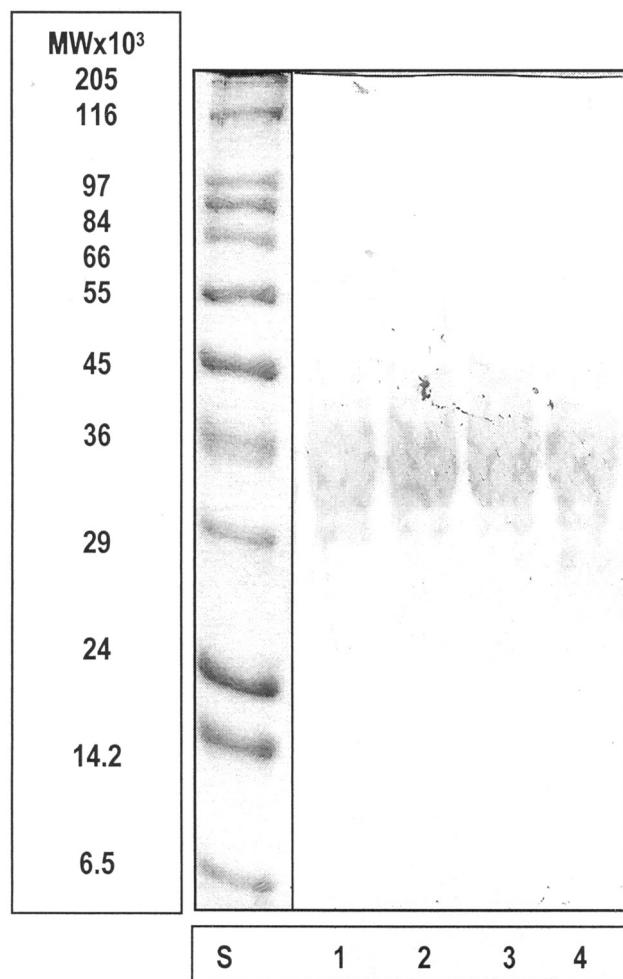


FIGURE 1. Immunoblotting analysis of gliadins extracted from non-germinated wheat grains cv. Tonacija.

1, 2 – non infected after 0 and 96 h of incubation; 3, 4 – infected with *F. graminearum* after 0 and 96 h of incubation; S – SDS-PAGE of molecular standards.

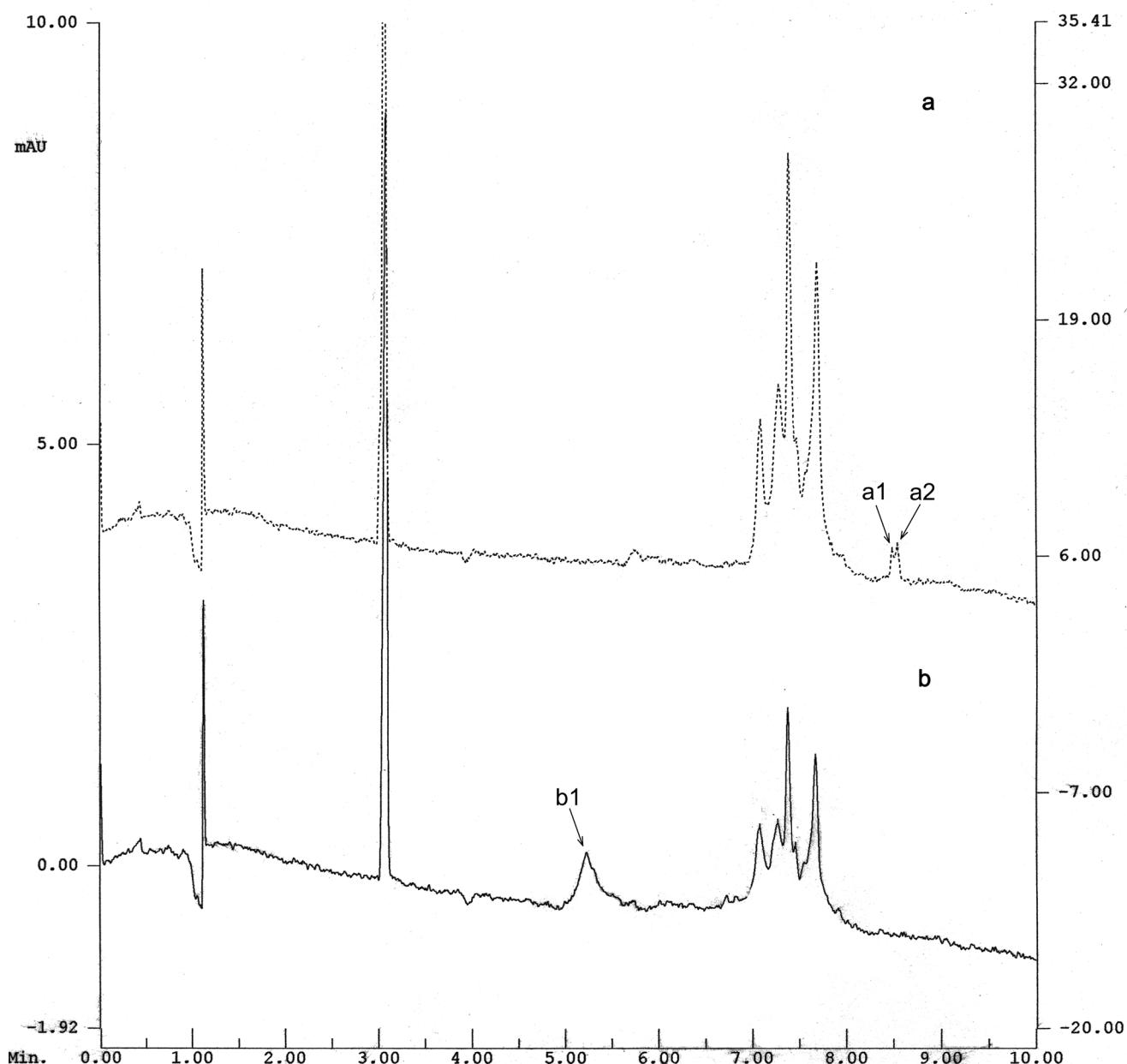


FIGURE 2. CGE electrophoregrams of gliadins extracted from wheat grains cv. Tonacja germinated, healthy (a) and infected with *F. graminearum* (b). Arrows a1, a2 and b1 indicate respectively hydrolysed sub-fractions with molecular weight of 74 kDa and 76 kDa and new sub-fraction with molecular weight of 15 kDa.

(Figure 1). The protein subfractions, reacting with antibodies from blood serum of patients with symptoms of food allergy to gluten, were characterised by molecular weights ranging from 29 kDa to 45 kDa.

Similar results were obtained by Mesa-del-Castillo *et al.* [2004] who investigated the immunoreactivity of soluble and insoluble fractions of storage proteins of wheat. The immunoreactive subfractions of water-insoluble proteins were characterised by molecular weights ranging from *ca.* 30 kDa to *ca.* 45 kDa. Hydrolysis of these proteins with the use of proteases (pepsin) produced the appearance of new subfractions with molecular weights of 25-30 kDa.

Effect of proteases of the *Fusarium* genus fungi on wheat proteins during germination of grain

Germination of kernels is a complex process that consists of a few phases requiring expression of multiple genes in different tissues. During the germination process, by absorbing water, the grain releases enzymes (proteases and amylases) hydrolyzing storage proteins and starch from the aleurone layer and epithelial scutellum. The beginning of digestion of storage proteins indicates that mechanisms protecting the storage proteins against hydrolysis during grain ripening – protease inhibitors – had ceased functioning [Müntz *et al.*, 2001]. During germination, proteins of a fungi-infected kernel are subject to hydrolysis by native proteolytic enzymes as well as by pro-

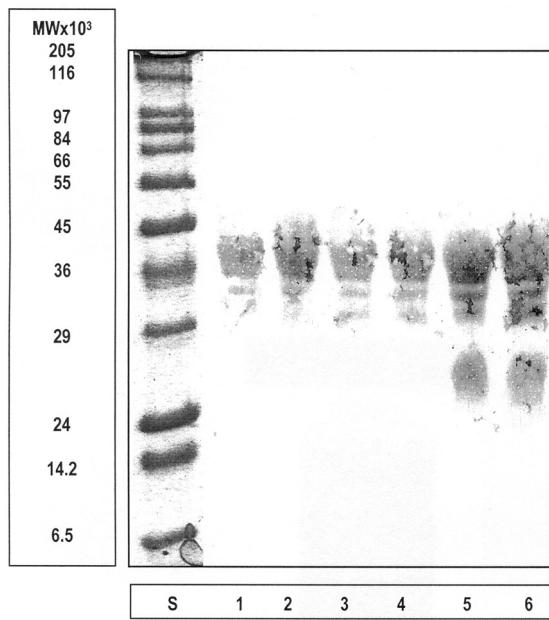


FIGURE 3. Immunoblotting analysis of gliadins extracted from germinated wheat grains cv. Tonacija. 1, 2, 3 – non infected after 0, 48 and 96 h of incubation; 4, 5, 6 – infected with *F. graminearum* after 0, 48 and 96 h of incubation; S – SDS-PAGE of molecular standards.

teases synthesized by pathogens [Müntz *et al.*, 2001; Jackowiak *et al.*, 2005]. Both the native and the fungal proteases may release allergenic peptides from storage proteins of wheat.

Our experiments demonstrated differences in the activity of endoproteases in non-infected grain and grain infected with *Fusarium graminearum*. At the beginning of germination, the endoproteolytic activity of the non-infected grain reached 0.66 U_{EA}/mg protein whereas that of the infected grain – 1.18 U_{EA}/mg protein. After 96-h germination, values of the endoproteolytic activity increased both in the case of infected and non-infected grain and accounted for 2.85 and 2.50 U_{EA}/mg protein, respectively.

The CGE analysis of molecular weights of prolamines fractions of non-infected wheat grain showed the presence of 7 protein subfractions with molecular weights ranging from 36 kDa to 76 kDa (Figure 2). The prolamines isolated from wheat grain subjected to germination and infected with *Fusarium graminearum* were characterised by molecular weights ranging from 36 kDa to 49 kDa. In the samples of infected grain, 2 subfractions of peptides with molecular weights from 74 kDa to 76 kDa were not detected, whereas there was a subfraction with a molecular weight of 15 kDa. In addition, the concentration of protein in particular subfractions of the analysed prolamines was higher by 5.0 to 17.0% in the samples of non-infected grain, as compared to respective subfractions of proteins in the samples infected with *Fusarium graminearum*.

The investigations of Wang *et al.* [2005] demonstrated that in wheat grain infected with *F. culmorum* the content of glutenin fractions (low- and high-molecular) decreased by 7.6 to 19.1%, whereas that of gliadins increased by 1.0 to 9.0%, as compared to the non-infected grain. These investigations also indicate the capacity of fungal proteases for hydrolysis of storage proteins

of wheat. Their activity results in a decrease in the sedimentation index of gluten, an increase in the concentration of free amino acids, and a decrease in the content of glutenin, especially its high-molecular fraction. The high activity of proteases in a wide range of acidity (from pH 4.5 to pH 8.5) and temperature (from 10°C to 100°C) contributes to degradation of storage proteins of wheat, especially endosperm proteins, during kneading and fermentation of dough [Wang *et al.*, 2005].

In contrast, experiments carried out by Dexter *et al.* [1997] did not demonstrate any qualitative changes in fractions of storage proteins of wheat grains infected with *Fusarium* and the non-infected ones. The *Fusarium* infected grains were, however, characterised by lower concentrations of a glutenin fraction as compared to the non-infected grains.

The immunological characteristics of proteins showed the presence of fractions reacting with antibodies from blood sera of patients with symptoms of food allergy to gluten in both the samples of germinating seeds infected with *Fusarium graminearum* and in the non-infected seeds (Figure 3). These subfractions were characterised by molecular weights ranging from 29 kDa to 45 kDa. In addition, the samples of wheat grain infected with fungi were found to contain immunoreactive peptides with molecular weights from 20 kDa to 24 kDa.

Studies by Rocher *et al.* [1995] confirmed the presence of immunoreactive subfractions of proteins among wheat prolamines. Their experiments demonstrated the presence of α -gliadin with molecular weights ranging from ca. 30 kDa and 45 kDa as well as γ -gliadin with molecular weights ranging from 30 kDa to 50 kDa that reacted with antibodies obtained from persons suffering from celiac disease.

Similar investigations carried out by Weiss *et al.* [1993] demonstrated the presence of allergenic subfractions among gliadins. Molecular weights of protein fractions reacting with antibodies of patients suffering from bakers' asthma ranged from 30 kDa to 45 kDa.

CONCLUSIONS

This study demonstrated that proteases synthesized by *Fusarium graminearum* affected the quantitative and qualitative composition of storage proteins of wheat. The released polypeptides, during germination of fungi-infected kernels react with antibodies isolated from blood of patients with diagnosed gluten intolerance. It seems, therefore, that fungal proteases, similar to native proteases of cereals kernels, are likely to release allergenic, or potentially toxic peptides and polypeptides.

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REFERENCES

1. Bechtel D.B., Kaleida L.A., Gaines R.L., Seitz L.M., The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem., 1985, 62, 191-197.

2. Bleukx W., Roels S.P., Delcour J.A., On the presence and activities of proteolytic enzymes in vital wheat gluten. *J. Cereal Sci.*, 1997, 26, 183-193.
3. Boyacioglu D., Hettiarachchy N.S., Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearum*. *J. Cereal Sci.*, 1995, 21, 57-62.
4. Dexter J.E., Marchylo B.A., Clear R.M., Clarke J.M., Effect of fusarium head blight on semolina milling and pasta-making quality of durum wheat. *Cereal Chem.*, 1997, 74, 519-525.
5. Gentile V., Violante V., D'amicco B., Illiano M., Luongo A., Tissue transglutaminase and coeliac disease pathogenesis: potential molecular mechanisms for other human diseases. *Neurochem. Int.*, 2002, 40, 79-83.
6. Jackowiak H., Packa D., Wiwart M., Perkowski J., Scanning electron microscopy of *Fusarium* damaged kernels of spring wheat. *Int. J. Food Microbiol.*, 2005, 98, 113-123.
7. Korhonen H., Pihlanto-Leppälä A., Rantamäki P., Tupasela T., Impact of processing on bioactive proteins and peptides. *Trends Food Sci. Tech.*, 1998, 9, 307-319.
8. Mesa-Del-Castillo M., Martínez-Cóceras C., Caballero M.L., Vazquez L., Moneo I., IgE binding to pepsin-digested water soluble and insoluble wheat proteins. *Allergy*, 2004, 59, 1229-1232.
9. Mills E.N.C., Madsen C., Shewry P.R., Wickers H.J., Food allergens of plant origin – their molecular and evolutionary relationships. *Trends Food Sci. Tech.*, 2003, 14, 145-156.
10. Müntz K., Belozersky M.A., Dunaevsky Y.E., Schlereth A., Tiedemann J., Stored proteinases and the initiation of storage protein mobilization in seed during germination and seedling growth. *J. Exp. Bot.*, 2001, 52, 1741-1752.
11. Nightingale M.J., Marchylo B.A., Clear R.M., Dexter J.E., Preston K.R., Fusarium head blight: effect of fungal proteases on wheat storage proteins. *Cereal Chem.*, 1999, 76, 150-158.
12. Pawelzik E., Permady H.H., Weinert J., Wolf G.A., Effect of *Fusarium*-contamination on selected quality criteria of wheat. *Getreide Mehl Brot*, 1998, 52, 264-266.
13. Pekkarinen A.I., Sarlin T.H., Laitia A.T., Haikara A.I., Jones B.L., *Fusarium* species synthesize alkaline proteinases in infected barley. *J. Cereal Sci.*, 2003, 37, 349-356.
14. Placinta C.M., D'mello J.P.F., Macdonald A.M.C., A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sci. Tech.*, 1999, 78, 21-37.
15. Prange A., Birzele B., Krämer J., Meier A., Modrow H., Köhler P., *Fusarium*-inoculated wheat: deoxynivalenol contents and baking quality in relation to infection time. *Food Control*, 2005, 16, 739-745.
16. Rocher A., Soriano F., Molina E., González-Limas G., Méndez E., Characterization of distinct α - and γ -type gliadins and low molecular weight components from wheat endosperm as coeliac immunoreactive proteins. *Biochim. Biophys. Acta*, 1995, 1247, 143-148.
17. Rollán G., De Angelis M., Gobbetti M., De Valdez G.F., Proteolytic activity and reduction of gliadin-like fractions by sourdough lactobacilli. *J. Appl. Microbiol.*, 2005, 94, 1495-1502.
18. Towbin H., Staehelin T., Gordon J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some application. *Proc. Natl. Acad. Sci. USA*, 1979, 76, 4350-4353.
19. Tsuji H., Kimoto M., Natori Y., Allergens in major crops. *Nutr. Res.*, 2001, 21, 925-934.
20. Wang J., Wieser H., Pawelzik E., Weinert J., Keutgen A., Wolf G., Impact of the fungal protease produced by *Fusarium culmorum* on the protein quality and breadmaking properties of winter wheat. *Eur. Food Res. Technol.*, 2005, 220, 552-559.
21. Weiss W., Vogelmeier C., Görg A., Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis*, 1993, 14, 805-816.
22. Zhou W., Kolb F.L., Riechers D.E., Identification of proteins induced or unregulated by fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). *Genome*, 2005, 48, 770-780.

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IMMUNOREAKTYWNE WŁAŚCIWOŚCI BIAŁEK ZAPASOWYCH PSZENICY OZIMEJ CV. TONACJA PORAŻONEJ GRZYBAMI *FUSARIUM GRAMINEARUM*

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Hodowla zbóż w niesprzyjających warunkach, czy niewłaściwe przechowywanie ziarna zbóż może doprowadzić do jego porażenia przez patogeny np. grzyby z rodzaju *Fusarium*. Infekcja ziarna zbóż przez grzyby z rodzaju *Fusarium* prowadzi do pojawienia się w nich toksyn, hormonopodobnych składników i enzymów m.in. proteaz.

Proteazy rodzime lub wytwarzane przez patogeny, hydrolizują białka ziarniaków zbóż. Uwalniane są peptydy i polipeptydy posiadające właściwości toksyczne i/lub alergizujące. U osób predysponowanych genetycznie może to prowadzić do wystąpienia chorób m.in. celiakii.

Przeprowadzone badania wykazały, że porażenie ziarna pszenicy *Fusarium graminearum* nie poddanego kielkowaniu nie zmienia właściwości immunoreaktywnych uwalnianych peptydów. Charakterystyka elektroforetyczna białek nie wykazała zmian w składzie ilościowym i jakościowym gliadyn.

Istotne zmiany stwierdzono w składzie białek gliadynowych izolowanych z kielkującego ziarna pszenicy skażonego *Fusarium graminearum*. Charakterystyka immunologiczna białek wykazała obecność polipeptydu o masie cząsteczkowej od 20 kDa do 24 kDa, reagującego z przeciwciałami uzyskanymi z krwi osób z nietolerancją glutenu, co wskazuje, że również proteazy fuzaryjne mogą uwalniać z białek zapasowych pszenicy potencjalnie niebezpieczne dla człowieka polipeptydy.