Potravinarstvo, vol. 8, 2014, no. 1, p. 82-86
doi:10.5219/340
Received: 11 February 2014. Accepted: 19 February 2014.
Available online: 7 May 2014 at www.potravinarstvo.com

ISSN 1337-0960 (online)

IMMUNOFLUORESCENT DETERMINATION OF WHEAT PROTEIN IN MEAT PRODUCTS

Michaela Petrášová, Eliška Zichová, Matej Pospiech, Bohuslava Tremlová

ABSTRACT

In food industry nowadays, there are various plant-origin protein additives which are meant for production of meat products. Among the most frequent additives of this type there are different kinds of flour, starch, fiber, and plant-origin proteins. Their usage at present is limited by the existing legislation not to prevent consumer deception but also for reasons of possible influence on consumer health. Therefore, this problem is paid a lot of attention not only in the Czech Republic but also all over the world. The main risk is seen in the impossibility to choose a suitable foodstuff for an individual prone to allergic reactions. Potential allergens are also often plant-origin raw materials which are added into foodstuffs for their technological qualities and low price. Wheat is widely cultivated cereal as well as an important source of proteins. After ingestion or inhalation, wheat proteins may cause adverse reactions. These adverse effects include a wide range of disorders which are dependent on the method of contact with wheat protein. These adverse effects can then take the form of various clinical manifestations, such as celiac disease, T-cell mediated inflammatory bowel disease, dermatitis, skin rash, breathing difficulties, allergy to pollen or to wheat flour or food allergy to foodstuffs containing gluten. The only possible protection against adverse immune reactions for those with food allergies is strictly excluding the allergen from their diet. Although the number of studies dealing with the reduction or loss of allergenicity is increasing, yet these practices are not common. Most of the population suffering from food allergies is thus still dependent on strict exclusion of foodstuffs causing adverse allergic reactions from their diet. In order to avoid misleading consumers and also to protect allergic consumers, analytical methods applicable to all types of foodstuffs have been developed. Unfortunately, detection of allergens in foodstuffs is relatively difficult because of the fact that they occur in trace amounts and are often masked by various parts of the product. This paper deals with detection of wheat protein in meat products bought in the retail network of the Czech Republic. Ten cooked meat products, especially types of sausages and soft salami which stated wheat protein in their composition, were examined. The samples were processed using the method of immunofluorescence and stained with Texas Red fluorochrome. The presence of wheat protein was demonstrated in all the examined meat products. From the results it follows that the method of immunofluorescence is suitable for detection of wheat protein in meat products.

Keywords: fluorescence microscopy; plant allergens; meat; adulteration; celiac disease

INTRODUCTION

Wheat protein is, inter alia, used in meat production where it contributes to the reduction of total production costs of meat products. On the other hand, wheat ranks among foodstuff ingredients given in **Directive 2003/89/EC** amending **Directive 2000/13/EC** as regards indication of the ingredients present in foodstuffs. Annex IIIa there to contain a list of ingredients of foodstuffs and products of them classified as possible allergens or substances with potential risk of intolerance. Food allergy is an abnormal immune response to foodstuffs **(Bruijnzeel-Koomen, et al., 1995)**. This type is characterized by an inappropriate reaction of one's immune system to the stimulus of the allergen which can be a protein or carbohydrate, for example **(Ferguson, 1992)**. In addition, food allergens contained in foodstuffs naturally are resistant to high temperatures, low pH in the stomach, and enzymatic digestion in the digestive tract **(Hefle, et al., 1996)**. However, it has been reported that there is no correlation between in vitro digestibility and protein allergy **(Fu, et al., 2002)**. Allergies to specific foodstuffs may in some cases exhibit also after ingestion of foodstuff of similar origin, which is known as a cross-reaction. This occurs when IgE antibodies originally produced against one allergen are produced also upon contact with a similar protein from another source **(Aalberse, et al., 2001)**. Food allergies have become a major health problem worldwide. Adverse health effects due to allergic reactions to food products or food ingredients occur in about 1-3% of population and in about 4-6% of children (including food intolerance). Food allergy is therefore more common in children than in adults. In recent years, wheat protein has also been more and more recognized as a cause of anaphylaxis due to foodstuffs **(Hischenhuber, et al., 2006)**. Sensitization to proteins of wheat grain may be caused in three different ways: inhalation, direct contact, and ingestion **(Battais, et all., 2006)**. Depending on the method of allergen exposure and based on immunological mechanisms, an allergy to wheat protein may exhibit as asthma and rhinitis, as well as contact urticaria or it may

occur as a classic food allergy affecting the skin, intestine and airways and is also known as anaphylaxis **(Sicherer, et al., 2000)**. Baker's asthma is one of the most common forms of occupational asthma. Baker's asthma is an occupational disease affecting 4-10% of bakery workers in European countries **(Baur, et al., 1999)**. Food allergy which is defined as an adverse immune response to food proteins, affects 6% of children and 3 to 4% of adults **(Sicherer and Sampson, 2000)**. Any protein from foodstuffs can cause an allergic reaction. In France between 2002 and 2004, wheat protein was responsible for 6% of anaphylaxis occurrences caused by foodstuffs **(Moneret-Vautrin, et al., 2005)**. In Iran, wheat protein is considered to be the most frequent trigger of anaphylaxis in children **(Pourpak, et al., 2007)**. In France, wheat ranks the eighth out of all allergies to foodstuffs in children and it ranks the twelfth in adults **(Rance et al., 1998)**. Allergy to wheat protein more often occurs in northern than southern Europe **(Rasanen et al., 1994)**. Data on the percentage of allergies to wheat protein vary. In his study, **Sicherer, et al., (2000)** states that 20% of children population suffers from wheat allergy. In **Niggemann's study of 2001**, the number of 14% is reported. **Moneret-Vautrin, et al., (2003)** states that allergy to wheat protein affects 10.9% of children and 25% of adults. On the other hand, in an American study focused on allergies in children, only 2.5% of children suffered from allergies to wheat protein **(Rance, et al., 2005)**. These numbers could have been underestimated because they represent only the most serious cases when hospital treatment was necessary. Clinical symptoms of wheat allergies are similar to those of other allergies to foodstuffs, with signs occurring in the skin, digestive system, and respiratory system **(Sicherer, 2000)**. The main symptom in children is atopic dermatitis, occurring either independently or in connection with respiration symptoms and digestion problems **(Moneret-Vautrin, et al., 2000)**. Various clinical symptoms were identified in adults, such as angioedema or eosinophilic esophagitis **(Scibilia, et al., 2006)**. Anaphylactic reaction caused by the contact with wheat protein was demonstrated in adults as well as in children **(Lehto, et al., 2003)**. For an allergic consumer it is especially important to know the exact composition of a foodstuff, especially as far as the presence of wheat protein is concerned. Analytical methods for the detection of allergens require high sensitivity, specificity, and sufficient robustness **(Battais, et al., 2006)**. As it follows from the paper of **Talandová, et al., (2013)** where the method of immunofluorescence was successfully applied to the products containing wheat protein, this method seems to be sufficiently sensitive and specific. Article focused an identity wheat protein in meat products.

MATERIAL AND METHODOLOGY

10 cooked meat products were examined, mainly types of sausages and soft salami bought in the retail network of the Czech Republic which, with respect to their composition, were supposed to contain wheat in various forms, such as wheat starch or protein, or they were labeled with a note saying "This product can contain traces of wheat". Four samples sized 1 cm^3 were taken from each meat product and then frozen. The samples were subsequently processed at the Department of Vegetable Foodstuffs Hygiene and Technology at FVHE (Faculty of Veterinary Hygiene and Ecology), UVPS Brno. The samples were cut into 4-μm-thick sections using HM 550 cryostat (Germany, Microm). These sections were then transferred to Thermo Superfrost slides (Germany, Thermo Scientific). Nine sections were cut for each meat product. Each sample was represented by four frozen blocks. From these blocks, microscopic sections were taken with trimming after 50 μm each. Immunofluorescent microscopy was selected as the method of detection as it is more sensitive and more selective than histochemical methods. The immunofluorescent procedure itself was started by inserting the sections into acetone. After the preparations were rinsed in PBS (phosphate buffer saline) for the period of 5 minutes, the blocking of endogenic peroxidase was carried out using a 3% solution of hydrogen peroxide. After repeated rinsing in PBS (2 x 5 min), the samples were placed into a wet chamber where the blocking of non-specific binding ran for 30 minutes using Goat Diluent Normal serum (GB, Vector Laboratories). Then the biotinylated primary antibody Anti-wheat (GB, Sigma-Aldrich A1052) was applied to the sections and the wet chamber was left in the fridge overnight. On the following day, the samples were rinsed in PBS (2 x 5 min). Then they were placed again into a wet chamber and secondary antibody (GB, Vector Laboratories BA-1000) was applied to the sections for the period of 30 min at room temperature. Rinsing in PBS (2 x 5 min) and application of fluorochrome followed. The Texas Red (GB, Vector Laboratories) was used as fluorochrome. Then the sections were mounted and examined using Leica DM3000 fluorescence microscope (Germany, Leica), and they were further processed with the help of Leica IM50 program (Germany, Leica). Nine sections were examined in this way for each meat product with the magnification of 40x and 100x.

RESULTS AND DISCUSSION

Meat products, on the cover of which their producers declared the use of wheat protein, were bought in the retail network and examined. Wheat, together with cow milk, eggs, soybean, nuts and peanuts, fish, shellfish, crustaceans and molluscs, is the cause of approximately 90% of allergies to foodstuffs and it is also the primary foodstuff causing anaphylaxis **(Sicherer and Sampson, 2000)**. In the interest of consumer protection, the European Commission issued **Directive 2003/89/EC amending Directive 2000/13/EC** as regards indication of the ingredients present in foodstuffs. Annex IIIa thereto contains a list of ingidients in foodstuffs and products of them classified as possible allergens or substances with potential risk of intolerance, such as gluten-containing cereals, crustaceans, eggs, fish, peanuts, soybean, milk (including lactose), nuts, celery, mustard, sesame seeds, sulphur dioxide and sulphites. **Directive 2003/89/EC** demands that each of the twelve above stated potentially allergenic constituents is labeled even if it forms less than 25% of the foodstuff. Therefore, the objective of this study was to verify a method suitable for demonstration of gluten in meat products.

Table 1 Wheat protein detected for individual measurements

Meat Product	Producer's Declaration	Block			
		A	B	C	D
1	**content of wheat protein**	+++	++	++	++
2	**content of wheat protein**	++	++	++	+++
3	**traces of wheat**	+	+	++	++
4	**content of wheat**	++	++	+++	+
5	**traces of wheat**	++	++	++	++
6	**content of wheat starch**	+	+/-	+	++
7	**content of wheat**	+++	+	++	++
8	**content of wheat protein**	++	+++	++	++
9	**content of wheat**	+	+	++	+++
10	**traces of wheat**	++	+	++	+

Explanatory notes: + to +++ shows the power of fluorescence intensity of wheat protein, +/- shows a dubious result.

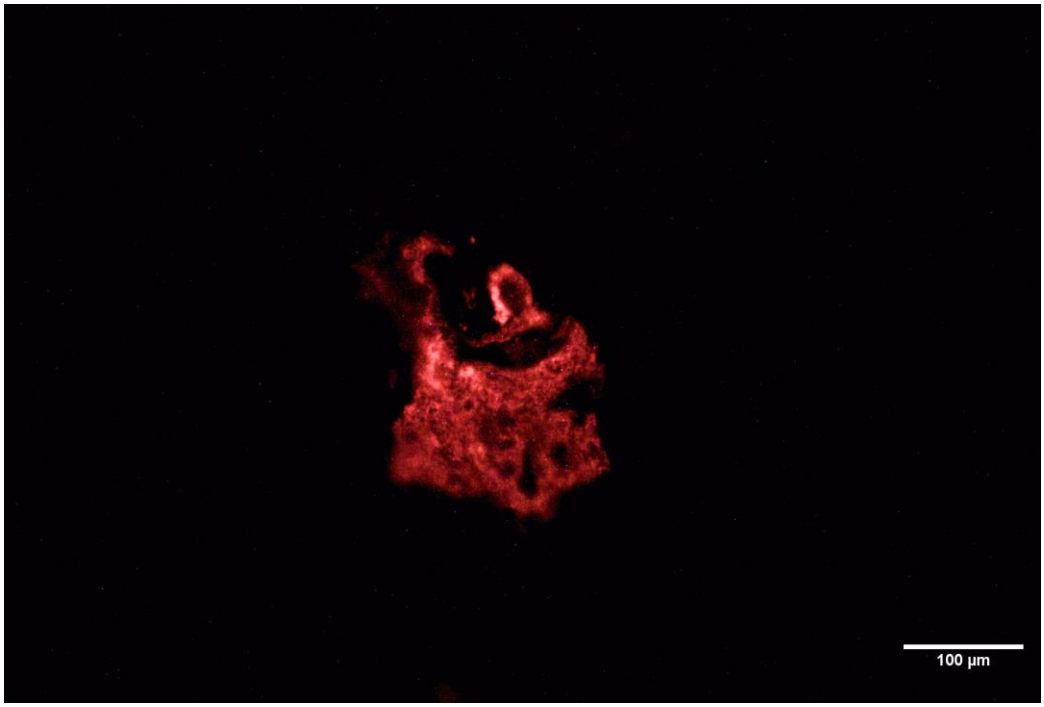

Figure 1 Sample no. 4: Wheat protein (red) in a meat product (black), fluorescence intensity +++, magnification 200x

Fluorescent immunohistochemistry was selected as the examination method. Wheat protein was detected on the basis of its specific structure and with the help of fluorescence which was created using the binding of Texas Red fluorochrome and excitation of light at the wavelength of 596 nm (Fig. 1). The results are given in Table 1. The number of crosses represents the power of fluorescence intensity of wheat protein. From the table it follows that wheat protein was detected in all the blocks for each meat product analyzed. From the results it follows that it is possible to confirm the presence of wheat protein using the method of immunofluorescence in products with targeted addition of wheat protein for improving texture and binding qualities which were labeled with "content of wheat protein" by the producer. The method of immunofluorescence was also sensitive for meat products labeled "traces of wheat" and the use of wheat was confirmed in all the products labeled in this way. For the meat product number 6, a dubious result was found in block B, which might have been caused by the low amount of gliadin due to the use of wheat starch.

CONCLUSION

Wheat protein ranks among allergens in foodstuffs which represent a serious health problem. The correct detection of the presence of these allergens in foodstuffs is absolutely necessary from the allergic consumer's point of view. Fluorescent immunohistochemistry could be used as one of these methods. With the help of this method, 10 meat products, for which the producers declared the presence or traces of wheat, from the retail network were examined. The presence of wheat protein was confirmed in all of these products. The method of immunofluorescence is therefore a sensitive method suitable also for the analysis of meat products for the presence of wheat protein. The results also show that producers are responsible in declaring the presence of the allergen in their products.

REFERENCES

Aalberse, R. C., Akkerdass, J., Van Ree, R. 2001. Cross-reactivity of IgE antibodies to allergens. *Allergy*, vol. 56, no. 6, p. 478-490. http://dx.doi.org/10.1034/j.1398-9995.2001.056006478.x PMid:11421891

Battais, F., Richard, C., Jacquenet, S., Denery-Papini, S., Moneret-Vautrin, D. A. 2008. Wheat grain allergies: an update on wheat allergens. *European Annals of Allergy and Clinical Immunology*, vol. 40, no. 3, p. 67-76. PMid:19334370

Baur, X. 1999. Baker's asthma: causes and prevention. *International Archives of Occupational and Environmental Health*, vol. 72, no. 5, p. 292-296. http://dx.doi.org/10.1007/s004200050377 PMid:10447658

Brujinzell-Koomen, C., Ortolani, C., Aas, K., Bindslev-Jensen, C., Bjorksten, B., Moneret-Vautrin, D., Wutrich, B. 1995. Adverse reactions to food. *Allergy, European Academy of Allergology and Clinical Immunology*. vol. 50, no. 8, p. 623-625. http://dx.doi.org/10.1111/j.1398-9995.1995.tb02579.x

Ferguson, A. 1992. Definitions and diagnosis of food intolerance and food allergy: consensus and controversy. *Journal of Pediatrics*, vol. 121, no. 5, p. 7-11. http://dx.doi.org/10.1016/S0022-3476(05)81400-3

Fu, T. J., Abbott, U. R., Hatzos, C. 2002. Digestibility of food allergens and nonallergenic proteins in stimulated gastric fluid and stimulated intestinal fluid: a comparative study. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 24, p. 7154-7160. http://dx.doi.org/10.1021/jf020599h PMid:12428975

Hefle, S. L., Nordlee, J. A., Taylor, S. L. 1996. Allergenic foods. *Critical Reviews in Food Science and Nutrition*, vol. 36, p. S69-S89. http://dx.doi.org/10.1080/10408399609527760 PMid:8959379

Hischenhuber, C., Crevel, R., Jarry, B. 2006. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. *Alimentary Pharmacology & Therapeutics*, vol. 23, no. 5, p. 559-575. http://dx.doi.org/10.1111/j.1365-2036.2006.02768.x PMid:16480395

Lehto, M., Palosuo, K., Varjonen, E. 2003. Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. *Clinical & Experimental Allergy*, vol. 33, no. 1, p. 90-95. http://dx.doi.org/10.1046/j.1365-2222.2003.01568.x PMid:12534555

Moneret-Vautrin, D. A., Kanny, G., Guerin, L., Flabbee, J., Lemerdy, P. 2000. The multifood allergy syndrome. *Journal of Allergy and Clinical Immunology (Paris)*, vol. 32, no. 1, p. 12-15. PMid:10723530

Moneret-Vautrin, D. A., Kanny, G., Périer, P. 2003. Prospective study (199-2002) of wheat flour allergy in children and adults, with reference to celiac disease. Relationship of DR1 allele to allergy in children. *Alim. Inter*, vol. 8, p. 2-8.

Moneret-Vautrin, D. A., Morisset, M., Flabbee, J., Beaudouin, E., Kanny, G. 2005. Epidemiology of life-threatening and lethal anaphylaxis: a review. *Allergy*, vol. 60, no. 4, p. 443-451. http://dx.doi.org/10.1111/j.1398-9995.2005.00785.x PMid:15727574

Niggemann B. 2001. The role of the atopy patch test (APT) in diagnosis of food allergy in infants and children with atopic dermatitis. *Pediatric Allergy and Immunology*, vol. 12, no. 14, p. 37-40. http://dx.doi.org/10.1034/j.1399-3038.2001.121408.x PMid:11380896

Pourpak, Z., Ghojezadeh, L., Mansouri, M., Mozaffari, H., Farhoudi, A. 2007. Wheat anaphylaxis in children. *Immunological investigations*, vol. 36, no. 2, p. 175-182. http://dx.doi.org/10.1080/08820130600941211 PMid:17365018

Rasanen, L., Lehto, M., Turjanmaa, K., Savolainen, J., Reunala, T. 1994. Allergy to ingested cereals in atopic children. *Allergy*, vol. 49, no. 10, p. 871-876. http://dx.doi.org/10.1111/j.1398-9995.1994.tb00790.x PMid:7535982

Rance, F., Grandmottet, X., Grandjean, H. 2005. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clin. Exp. Allergy*, vol. 35, no. 2, p. 167-172. http://dx.doi.org/10.1111/j.1365-2222.2005.02162.x PMid:15725187

Rance, F., Kanny, G., Dutau, G., Moneret-Vautrin, D. 1998. Aspects cliniques de l'allergie alimentaire. *Rev. Fr. Allergol*, vol. 38, p. 900-905. http://dx.doi.org/10.1016/S0335-7457(98)80160-3

Regulation (EC) No 2000/13 of 20 March 2000 on the approximation of the laws of Member States relating to the labeling, presentation and advertising of foodstuffs. OJ L 109, 06.05.2000, p. 0029-0042.

Regulation (EC) No 2003/89 of 10 November 2003 amending Regulation 2000/13/EC as regards indication of the

ingredients present in foodstuffs (Text with EEA relevance). OJ L 308, 25.11.2003, p. 0015-0018.

Regulation (EC) No 2006/142 of 22 December 2006 amending Annex IIIa to Directive of the European Parliament and Council regulation 2000/13/EC, listing the ingredients which must under all circumstances appear on the labeling of foodstuffs (Text with EEA relevance). OJ L 368, 23.12.2006, p. 110-111.

Scibilia, J., Pastorello, E. A., Zisa, G. 2006. Wheat allergy: a doubleblind, placebo-controlled study in adults. *Journal of Allergy and Clinical Immunology*, vol. 117, no. 2, p. 433-439. http://dx.doi.org/10.1016/j.jaci.2005.10.014 PMid:16461145

Sicherer, S. H. 2000. Determinants of systemic manifestations of food allergy. *Journal of Allergy and Clinical Immunology*, vol. 106, no. 5, p. S251-257. http://dx.doi.org/10.1067/mai.2000.110158 PMid:11080740

Sicherer, S. H., Morrow, E. H., Sampson, H. A. 2000. Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, vol. 105, no. 3, p. 582-586. http://dx.doi.org/10.1067/mai.2000.104941 PMid:10719311

Sicherer, S. H., Sampson, H. A. 2000. Peanut and tree nut allergy. *Current Opinion in Pediatrics*, vol. 12, no. 6, p. 567-573. http://dx.doi.org/10.1097/00008480-200012000-00010 PMid:11106277

Talandová, M., Zichová, E., Pospiech, M., Tremlová, B. 2013. *Využití fluorescenční mikroskopie k detekci pšeničného proteinu v modelových masných výrobcích*. Mladí vedci. Bratislava: Ministerstvo pôdohospodárstva a rozvoja vidieka SR, p. 114. ISBN-978-80-970552-8-8.

Acknowledgments:

This work was supported by the grant of IGA 2/2012/FVHE.

Contact addresses:

Michaela Petrášová, University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Vegetable Foodstuffs Hygiene and Technology, Palackého tř. 1/3, 612 42 Brno, Czech Republic, E-mail: petrasovam@vfu.cz.

Eliška Zichová, University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Vegetable Foodstuffs Hygiene and Technology, Palackého tř. 1/3, 612 42 Brno, Czech Republic, E-mail: eliska.zichova@seznam.cz.

Matej Pospiech, University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Vegetable Foodstuffs Hygiene and Technology, Palackého tř. 1/3, 612 42 Brno, Czech Republic, E-mail: pospiechm@vfu.cz.

Bohuslava Tremlová, University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Vegetable Foodstuffs Hygiene and Technology, Palackého tř. 1/3, 612 42 Brno, Czech Republic, E-mail: tremlovab@vfu.cz.