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Study on properties of components triggering hypersensitivity reactions using real food matrices

Summary of PhD thesis

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1 Introduction and goals

Hypersensitivity reactions (allergy, coeliac disease) triggered by food components pose a serious food safety issue. The principal treatment of food allergy is the total avoidance of allergic food components but the implementation of this can be difficult.

Certain proteins of food components are responsible for inducing symptoms. Binding of the allergens (antigens) to the antibodies is taking place through so-called epitopes. The epitopes are immune reactive parts of proteins which are recognized by the antibodies. Different food components have various protein compositions, contain more proteins which have epitopes triggering hypersensitivity reactions and on the surface of an antigen generally more epitopes can be found that take part in the immune reactions independently. Besides, as effects of production processes in foods, the proteins can interact with other proteins and other components of the food matrix. During different processes, the immune dominant epitopes can be modified which can potentially affect the parts of proteins inducing adverse physiological effects. However, the current scientific literature is not reporting unambiguous opinions in this topic. The number of allergenic/toxic proteins, the epitope sequences of individual proteins and the behaviour of proteins are debated questions.

Quantification of components triggering hypersensitivity reactions in foods is an important issue from food safety and economic point of views. The availability of reliable food products and the appropriate information are essential for the safety of allergic or coeliac patients. For this, appropriate technology in the production of foods and reliable analytical methods for their monitoring are necessary. For determination and quantification of triggering proteins, several methods are available. However, neither reference methods nor generally accepted certified reference materials are in service. This lack makes the validation of the methods more difficult. In routine analysis, the ELISA method became general because of its high specificity and easy handling. However, the commercially available ELISA kits can differ significantly. Target components can vary, for example in case of ELISAs developed for the detection of the presence and quantification of milk proteins there are kits using caseins and/or β-lactoglobulin as target protein(s). Beside the target proteins, the applied extraction solutions, the methodology of sample preparation, the materials used for calibration and the analytical performance characteristics can also be different. Due to these reasons, the ELISA tests developed for the quantification of the same component provide different results.

Food matrices often contain more than one allergenic compound, thus a relatively new way of method development is the development of analytical methods for simultaneous

detection of multiple components. Thus, it is obvious that there is a demand on production of model matrices which can give the possibility for the investigation of several allergens simultaneously and for validation of the related methods.

The basis of research at our Department was the development of model products which contain milk or egg or gliadin proteins in defined amounts and in a processed food matrix. In the course of this work, the recipe of the model products and the methodology of their production were developed. The procedure ensures the homogeneous distribution of reference milk powder, reference egg powder and gliadin isolate applied as allergen sources in low (ppm) amount in the matrix. Sampling from every steps of the production process enabled the investigation of the effects of processes (mainly heat treatment) on the measurable allergen content. It could be observed that heat treatment (baking) significantly reduced the measurable protein concentration. Reference material candidate model products containing gliadin developed by our research group gave the possibility for the first time for a comparison of commercial gluten ELISA methods under controlled circumstances. The results of this study clearly showed that the analytical results of ELISA kits vary in a broad range. In case of native proteins, the recoveries were between 60 and 600 %, while in heat treated matrices between it was found to be between 15 and 400 %. These preliminary results point to the extent and nature of the inaccuracy of the currently applied ELISA methods, and in certain cases to the source of the errors as well. The extent of the revealed errors is significant, mostly when the regulation for allergens requires zero tolerance and 20 and 100 ppm threshold in case of gluten. As a result, this issue jeopardizes the safety of patients, discredits food safety, and makes controlling and sanctions more difficult. In other words, this uncertainty affects the activity of all stakeholders in this field. Due to this reason, identification of the revealed errors and improvement of analytical methods accordingly are key questions.

In order to solve these problems, the main goals of this PhD work were the following:

- Investigation of the effects of food processing steps – hydration, heat treatment –on the target proteins. Clarifying the background of the decrease in the measurable protein concentration with ELISA methods.
- Investigating the effects of other macro components of the food matrix on the quantification of allergenic proteins.
- Development and characterization of multi component (milk, egg, gluten, soy) processed food model products.

- Stability study of reference material candidate model products.

2 Materials and methods

During our research work, new model products were developed and characterised which contained milk, or egg or soy or gliadin individually or the four components simultaneously. The investigations were carried out using samples from all three production phases: powder mixture of raw materials, raw dough and heat treated (baked) product. The basis of the model product development was the production process of the reference material gluten containing (made with PWG gliadin isolate) developed during the doctoral work of Zsuzsanna Kormosné Bugyi (2012)[1]. In the present study, development of new model products was performed that contain the four most common allergens of our country (milk, egg, soy, and gliadin) individually or simultaneously. As this activity was part of the research-development work, the results of this working phase will be presented in chapter 3.1 in detail. For the production of model matrices, reference materials (IRMM-BCR 380R reference milk powder, NIST 8415 reference egg powder, NIST 3234 reference soy flour, PWG gliadin isolate[2]) were used as allergen sources. Although these materials have certified chemical composition data, they are not materials for allergen analysis. A part of raw dough and baked cookie model products was defatted in order to examine the effects of sample preparation and matrix components on the analytical results.

For the ELISA measurements the R-Biopharm Ridascreen Fast Milk, Ridascreen Fast Ei/Egg Protein, Ridascreen Fast Soya, Ridascreen Gliadin and the Romerlabs AgraQuant Casein, AgraQuant Egg white, AgraQuant Soy, AgraQuant Gluten G12 kits were used. The protein profile and subunit composition of model products were examined using SDS-PAGE, Lab-on-a-chip and SE-HPLC. The measured results were statistically evaluated with the investigation of the means and standard deviations, with carrying out F- and t-tests and with analysis of variance (ANOVA). The results of the 2-, 3- and 4-factor ANOVAs were used for the quantification of analytical error components.

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[1]Kormosné Bugyi Zsuzsanna (2012): Improving the conditions of the analytical methodology for the quantification of food allergens. PhD thesis.

[2]van Eckert R., Berghofer E., Ciclitira P.J., Chirdo F., Denery-Papini S., Ellis H.J., Ferranti P., Goodwin P., Immer U., Mamone G., Mendez E., Mothes T., Novalin S., Osman A., Rumbo M., Stern M., Thorell L., Whim A., Wieser H. (2006): Towards a new gliadin reference material–isolation and characterisation. Journal of Cereal Science, 43: 331-341.

3 Results and discussion

3.1 Development of model products

The PWG gliadin isolate was changed to standard wheat flour in the model products in order to examine the effects of allergenic source change with ELISA method and to investigate the effects of heat treatment with size-exclusion chromatography (SE-HPLC). Protein and dry gluten content of the wheat flour were used to define the needed amount of flour for 10 and 50 ppm gliadin content and for 2 % protein content in the model products.

For the investigations of matrix effects, single and multi component model products were developed with commercially available gluten-free flour (which is not easy to standardize) and with amaranth flour (which is considered to be gluten-free) as well.

In order to examine the composition changes of applied protein sources during processing with the available analytical methods (electrophoretic techniques), a new model products had to be produced. This new product did not contain other, interfering proteins, while the concentration of the proteins to be analyzed was increased according to the sensitivity of these methods. Single and multi component matrices were produced with corn starch of a confirmed allergen-free status. The protein concentrations were 2 % for all components.

In all three cases, we developed production procedures and homogenizing methodologies for these new model matrices. The homogeneity of the protein distribution in the matrices was checked with ELISA method, while in case of the starch based products, total protein determinations were carried out. Statistical evaluation of the results showed that all sample matrices have physico-chemical characteristics and homogeneity suitable for the research goals. The corn starch based products can also play an important role in a later reference material development work.

3.2 Investigation of matrix effects

A study was carried out to compare the ELISA results of the gluten-free flour and the amaranth flour based model products. The results showed that the protein recoveries differ considerably: the amaranth flour based matrices show significantly lower measurable concentrations in case of all four proteins. Physico-chemical characteristics and protein profile of the applied flours are different so it can happen that the allergenic proteins formed interactions with the components of the amaranth flour which modified their solubility properties, extractability and the affinity to the applied antibody. Thus, the reason of the differences is probably in these deviations.

Furthermore, the effects of lipids as matrix components were studied on the quantification of proteins by introducing a defatting step before the ELISA sample preparation. In the milk or gliadin containing matrices the recovery increased significantly after defatting. In case of egg and soy the recovery was variable in the different type of samples, moreover, the differences were not always significant and both increases and decrases have been observed. Thus, it seems that the effect of defatting is not generalizable, depends on the type of the target protein, on the applied sample preparation methodology and on the type of the applied ELISA test as well. So our results indicate that the lipid extraction can influence the analytical result, especially in case of samples with high lipid content. The result may seem to be obvious since, except gliadin, water phase extraction of proteins is applied by the studied kits. However, defatting as a sample preparation step is not advised by any of the kits in routine analysis. Relying upon these results we suggest the use of defatting as sample preparation step in order to improve the accuracy of quantifications in case of foods with high lipid content.

The third type of the matrix effect studies was the modification of the complexity of the applied protein source which models the real products better. The use of sample matrix/reference material in which the added component is originated from protein isolate is easier to handle from the analytical point of view. In our studies the PWG gliadin isolate was suitable for this requirement. However, real food samples mostly contain wheat flour or proteins – and other components – originated from wheat fractions. We wanted to investigate the identifiable effects of these differences in the analytical results with the help of the model products made with gliadin isolate and wheat flour. The results show that the application of wheat as gliadin source led to higher measured concentration than the model products made with gliadin isolate. These deviations were significant in case of every sample type. It is clearly seen that while the measurable concentrations of the model products made with the isolate were near or below the theoretical concentration, in case of wheat flour the concentrations were considerably higher. These results draw attention to one of biggest problems of the present allergen analysis, namely the issue of the analytical accuracy and as a consequence of this to the weak points of the current regulation. These analytical errors can be quantifiable in case of gluten/gliadin which was already carried out with the help of the developed model products. At the same time, the phenomenon is present for all critical proteins and protein sources.

Finally, the most characteristic food composition situation was modelled with the investigation of the presence of more allergenic proteins. The results show that in case of

certain samples differences could be observed between the analytical results of the single and the multi matrices. It also could be observed that no unambiguous trends could be established for the recoveries and standard deviations in case of either the raw material or the applied kit or the processing level; the effects are not significant in most cases.

As the results of comparison of single and multi matrices and investigation of the raw material modification come from the same experiment, it was possible to evaluate the effects of these factors in one complex statistical analysis. During this, determination of deviation components was carried out. With these components it can be identified to what extent were the analytical result influenced by the examined factor (in this case, the use of different raw materials and the simultaneous presence of several allergen components) and the analytical errors. Based on the analysis it can be said that the variation of the measured concentrations is mostly affected by the choice of the raw material. This effect leads to remarkable differences in the measured values, which outweighs the observed difference between single and multi component matrices.

The trials performed during my doctoral work draw attention to the difficulties caused by foods with variable composition. However, development and investigation of further model products with different compositions are necessary for better understanding of the behaviour of the allergenic proteins.

3.3 Effects of food processing

During food processing different environmental effects have an influence on food components. The most typical processes are heat treatment, pH or ion strength change, high pressure or enzymatic treatments. Protein denaturation due to heat treatment is a well-known phenomenon that can affect the analytical results of the allergen components through structural and composition changes in the protein molecules, complex formation (combined proteins) resulted from chemical reactions and changes of the epitopes. The phenomenon is discussed in the scientific literature but the extent of the effects influencing the analytical results and the amount of knowledge that could help to understand the background, mostly for particular proteins, are limited.

It can be said for all studied components that a small decrease could be observed in the ELISA results of raw dough samples compared to the powder mixtures. In contrast, a major, statistically significant decrease could be experienced in the measurable protein concentration after heat treatment.

Since the effects of processing on the ELISA results were investigated in the single and multi allergen containing matrices with gluten-free flour and amaranth flour also, a complex statistical evaluation of these three factors was possible. The largest share of the result fluctuation comes from the level of processing in case of milk, egg and soy containing model products. At the same time, in gliadin matrices the applied raw material had the major impact. This can be explained by the lower decrease in the measurable concentration due to heat treatment in gliadin compared to the other components because gliadin proteins are more heat stable.

Furthermore, we investigated the deviations between the ELISA kits suitable for the quantification of the same components but originated from different producers and applying partly different target proteins. In general, it can be concluded that differences could be observed between the results of ELISA kits used for the quantification of the same analyte. These differences are significant in some cases (e.g. matrices containing milk powder or soy flour) however the deviations depend also on the nature of the examined matrix (native protein or heat treated matrix). Besides, with a few exceptions (e.g. dough containing soy flour) the measured concentrations of all kits show significant difference from the theoretical concentration as well.

For the statistical verification of these findings and for estimating the extent of factor effects, a 4 factor analysis of variance was carried out. The four factors (raw material, number of allergen sources, level of processing and the applied ELISA kit) were tested in one experimental line. It could be observed that the applied kit has a great influence on the analytical result; in case of milk and gliadin this provides the largest share of the standard deviations. According to our experimental results it can be concluded that the determination of all four components is affected by the matrix components and the processing steps, however, the extent of the effects depends on the analytes and the applied analytical method.

Thus, the concentration changes measured by ELISA tests during food processing are statistically proven. It can be seen that the nature of the effects is the same; however, their extent depends on the protein source. For the examination of the background of these phenomena, electrophoretic studies were performed. It is clearly seen from the SDS-PAGE and LOC results that the protein patterns of powder mixtures and raw dough samples did not differ significantly. In contrast, on the gel picture of the heat treated products the low molecular weight proteins cannot be identified and the intensity of high molecular weight protein bands reduced significantly. In order to increase protein solubility, some ELISA tests and the electrophoretic methods generally apply reducing agents. In most cases, the extraction

with reducing agents improved the solubility of proteins and their identification on the gels but did not mean a perfect solution in the improvement of protein solubility as certain protein subunits were missing in the heat treated products. Large aggregates and complex proteins formed by heat treatment cannot be solubilised with this procedure in a way that would have improved the analytical results. In case of gliadin, this observation was verified by SE-HPLC measurements as well. A relationship could be revealed between the changes of ELISA results and the decrease of the amount of soluble proteins measured by SE-HPLC. The extent of the gliadin content decrease measured by ELISA is similar to the extent of changes of chromatographic peak areas.

Thus, it is clearly stated that solubility decrease originates primarily from protein denaturation and complex formation stands in the background of the measurable protein content decrease due to heat treatment measured by ELISA methods. Furthermore, structure changes of epitopes and consequently the analytical error originated from the immune activity (can) have substantially smaller effect in complex, processed (heat treated) matrices. This statement can be extended for all examined protein sources but of course the extent of the solubility changes differs in case of different proteins. It designates the ways of method developments in order to improve the protein solubility, namely the development of sample preparation which is paramount for the reliability of the analytical results. Therefore, such processes should be applied that are improving solubility of specific proteins and such target proteins should be selected which are less heat labile.

3.4 Stability study

The ultimate goal of this research is the development of certified reference materials which contain one or more allergens in defined amount in a real food matrix. The reference materials have to meet several requirements one of which is the certification of the stability of the given material. During my doctoral work, a stability and storage study was carried out in case of the model matrices containing gliadin. It was performed using three storage conditions (room temperature, refrigerator, freezer) for 12 months.

In case of the model products based on gluten-free flour, a continuous decrease could be observed in the measurable gliadin concentration, the recoveries decreased to 66 % at the end of the twelfth month. However, one of storage conditions did not show significant changes in the time according to the statistical analysis. The measured mean concentrations show great fluctuation in case of the cookies made with amaranth flour, the recoveries vary between 78 and 135 %. According to the statistical evaluation, the gliadin concentration changes

significantly in the room temperature and refrigerator stored samples over time. In contrast, cookies stored in a freezer showed no significant differences between different sampling dates.

3.5 Conclusions

In my dissertation, matrix effects and technological operations affecting the ELISA quantification of certain protein components and sources triggering allergy and celiac disease were studied and an attempt was made to explain the obtained results. For the implementation of the objectives, differently processed model products were developed that contained the four most commonly problematic protein sources (milk powder, egg powder, soy flour, gliadin) individually or simultaneously. The experimental products modelled the composition and physico-chemical properties of real food matrices. Thus, we have established an experimental environment in which the model products became suitable for execution of two directions of development simultaneously, namely the exploration of the analytical errors and the development of reference materials. We experienced that in most cases both protein and non-protein components affect the analytical results. The heat denaturation of proteins results in composition and structural changes causing principally solubility changes of the examined components. Compared to this, the effects of the other factors (e.g. denaturation of the epitopes and consequently the changes of the immunoreactivity) are negligible.

The experimental model product gave the possibility to estimate the nature and the extent of the factors influencing the analytical results as well. The experimental design compiled for this purpose and the statistical evaluation of the results clearly show that beside the technological operations (in this case heat treatment) the nature of the matrix (the characteristics and physico-chemical properties of the examined sample) and the applied method (ELISA kits produced by different manufacturers and using different sample preparation, target proteins and calculation methodologies) as experimental factors have a great impact on the analytical results. These factors cause errors exceeding the analytical uncertainty originated from replicate measurements. The extent of the single factors is different for the different allergenic components; the estimation of these was carried out with the applied analytical methodologies.

According to our results it seems to be proven that the presence of more allergenic proteins simultaneously – in the ppm range – has a smaller effect compared to the above mentioned factors so their effect is negligible. For the routine analysis, it means that during the usage of the currently applied test kits the presence of several allergens simultaneously in

foods means no risk for the accuracy of the analytical result. On the other hand, it became clear that our model matrices and their production procedure can be suitable for the production of multi component reference materials as well. According to the stability study of our model products, the matrices can be stored under refrigerated conditions for a longer period of time (at least one year) so theoretically they could be used for commercial distribution and utilization as reference materials as well.

Consequently, it seems clear that in case of processed foods the reliability of the results of the ELISA methods can be improved primarily by the development of sample preparation protocols and by the increase of the solubility of the target proteins. The issue of sample preparation is more difficult with respect of multi method development, because different proteins can be extracted by solvents with different composition. Thus, it is difficult to produce a universal solvent which is capable of extracting every protein from native and processed food matrices as well. In case of the immunoanalytical methods, the choice of the target protein is a key issue because their amount is affected by genetic and environmental factors which cause inaccuracy in the analytical results and in the calculation methodologies. But it is not clarified that the deviation of the results are originated from the application of different antibodies or from the differences in extraction efficiencies of different extraction solutions. However, studying the affinity of the antibodies is not possible without the improvement of the extraction solutions. So the solution of the sample preparation problems has primary importance in order to improve the reliability of the analytical results. Solution of the mentioned problems requires considerable efforts. At the same time, use of our model matrices as reference material candidates or internal material samples can give the opportunity to harmonize the analytical results of different methods, to clarify the background of the observed discrepancies and can help to realize method validation. It can also contribute to the improvement of the reliability of allergen analysis which is an important requirement from food safety, economical and regulatory points of view as well.

4 Theses

1. Laboratory scale production procedures for experimental matrices modelling the characteristics of real food product were established. The developed model products contain milk or egg or soy or gliadin proteins in a defined amount, in a low (ppm) concentration range applied in allergen analysis. These products make it possible to investigate the effects of matrix components and processing steps on the analytical data.

Furthermore, we suggested creating further model products, which can help to understand the ongoing processes in real food matrices (2, 4, 5).

2. Model matrices were develped that contain four allergenic components (milk powder, egg powder, soy flour and gliadin) simultaneously. Homogeneous distribution of components was assured in the products and the reproducible producing process was established. Presence of multi components did not cause significant effects on the quantification of the individual components. Thus, use of these model products as reference materials can be possible after further investigations (2, 6).
3. The effects of matrix components on the analytical results were investigated and it could be observed that the allergen sources, the raw materials and the lipid components affect the measurable allergen content. These effects should be taken into account during the development sample preparation (protein extraction) protocols and during the application of the model products as reference materials as well (3, 6).
4. Changes of protein subunit composition of allergen sources were followed in real food matrices during the processing steps. It could be observed that the protein subunit composition undergoes significant modifications, which leads to a decrease of protein solubility (1, 3).
5. In case of the four examined components triggering hypersensitivity reactions, the solubility of proteins changed to a different extent. The different solubility properties should be taken into account during the development of multi methods and multi component reference materials (1, 2, 6).
6. The effect of the applied ELISA methods on the analytical result was studied. Target proteins of ELISA tests applied for quantification of the investigated components are different and the extraction methods differ as well. So, the ELISA tests give different results in case of analysis of the same product. Thus, harmonization of ELISA methodology is necessary which can be supported by the usage of the experimental matrices as reference materials (2, 4, 6).

5 Application possibilities

With the help of the model products developed during this research, it becomes possible to better understand the components triggering hypersensitivity reactions. The model products can also be appropriate as reference materials. The developed production procedure gives the opportunity for production of further model matrices, which can help to understand the interactions of proteins and other food components. With the involvement of new model

products and the application of the statistical methodology established in this study, the estimation of uncertainty of the ELISA results could be improved. This information can support method development, can be utilized for the harmonization of the regulations and the analytical possibilities and can promote the harmonization of validation of the allergen analytical methodologies as well.

6 Publications

Publications related to the PhD thesis:

1. K Török, L Hajas, V Horváth, E Schall, Zs Bugyi, S Tömösközi (2014): Heat induced changes of proteins measured in allergen ELISA kits. *Acta Alimentaria*, elfogadva. IF: 0,475
2. K Török, V Horváth, Á Horváth, L Hajas, Zs Bugyi, S Tömösközi (2014): Investigation of incurred single- and multi-component model food matrices for determination of food proteins triggering allergy and coeliac disease. *European Food Research and Technology*, 239: 923-932. IF: 1,436
3. K Török, L Hajas, Zs Bugyi, G Balázs, S Tömösközi (2014): Investigation of the effects of food processing and matrix components on the analytical results of ELISA using an incurred gliadin reference material candidate. *Acta Alimentaria*. *DOI: 10.1556/AAlim.2014.0018* IF: 0,475
4. S Tömösközi, K Török, Zs Bugyi, L Hajas: Reference Materials for allergen testing. Eds. George Siragakis and Dimosthenis Kizis (2014): Food Allergen Testing: Molecular and Immunochemical and Chromatographic Techniques. *Wiley-Blackwell* 215-236. ISBN: 978-1-118-51920-2
5. K Török, Zs Bugyi, L Hajas, Zs Adonyi, S Tömösközi (2011): Az élelmiszerallergének mérésének lehetőségei ma- kihívások, megoldások, a fejlesztés irányai. *Élelmiszervizsgálati Közlemények*, 57 (2): 83-91. IF: 0,040

6. K Török, L Hajas, V Horváth, E Schall, Zs Bugyi, S Tömösközi: Investigation of analytical errors with statistical tools in case of ELISA-based allergen methods. *European Food Research and Technology*, beadva. IF: 1,436

Other publications:

Bugyi Zs, Török K, Hajas L, Adonyi Zs, Popping B, Tömösközi S. (2013): Comparative study of commercially available gluten ELISA kits using an incurred reference material. *Quality Assurance and Safety of crops & foods*, 5(1): 79-87. IF: 0,642

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Bugyi Zs, Török K, Hajas L, Tömösközi S. (2012): Egy mindennapi élelmiszer-alapanyag, a búza. Barát vagy ellenség? *Élelmiszer Tudomány Technológia,* 4: 5-9.

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Bugyi Zs, Nagy J, Török K, Hajas L, Tömösközi S. (2010): Towards development of incurred materials for quality assurance purposes in the analysis of food allergens. *Analytica Chimica Acta*, 672: 25-29. IF: 3,757

Oral presentations:

6th International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 5-8 November 2013

Kitti Török, Vanda Horváth, Lívia Hajas, Zsuzsanna Bugyi, Sándor Tömösközi: Towards a multi component incurred reference material for food allergen analysis

4th International MoniQA Conference, Budapest, Hungary 26 February – 1 March 2013

Kitti Török, Lívia Hajas, Zsuzsanna Bugyi, Sándor Tömösközi: Reference material candidate for gluten analysis- Exactness, stability, method comaprison, further development

11th European Young Cereal Scientists and Technologists Workshop, Barcelona, Spain, 9-11 May 2012

Kitti Török, Zsuzsanna Bugyi, Lívia Hajas, Tamás Langó, Ágnes Horváth, Sándor Tömösközi: Development of incurred reference material for gliadin quantification

3rd International MoniQA Conference, Varna, Bulgaria, 27-29 September 2011

Kitti Török, Zsuzsanna Bugyi, Lívia Hajas, Zsanett Adonyi, Tamás Langó, Sándor Tömösközi: Food allergens-the way towards method validation

Posters:

6th International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 5-8 November 2013

Kitti Török, Vanda Horváth, Lívia Hajas, Zsuzsanna Bugyi, Sándor Tömösközi: Towards a multi component incurred reference material for food allergen analysis

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Kitti Török, Attila Bagdi, Zsuzsanna Bugyi, Lívia Hajas, Tamás Langó, Zsanett Adonyi, Sándor Tömösközi: A study on properties of gliadin reference material candidate

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Kitti Török, Zsuzsanna Bugyi, Lívia Hajas, Zsanett Adonyi, Sándor Tömösközi: Challenges and solutions in reference material development for quantification of food allergens

10th European Cereal Scientists and Technologists Workshop, Helsinki, Finland, 23-25 May 2011

Kitti Török, Zsuzsanna Bugyi, Lívia Hajas, Zsanett Adonyi, Sándor Tömösközi: Cereal protein sensitivity, analysis, regulation

Hungalimentaria 2011, „Élelmiszer- és takarmányellenőrzés: Gyorsabban-Pontosabban-Biztonságosabban", Budapest, Hungar, 19-20 April 2011

Török Kitti, Bugyi Zsuzsanna, Hajas Lívia, Adonyi Zsanett, Tömösközi Sándor: Gliadin meghatározására alkalmas ELISA módszerek összehasonlítása, a validálás feltételrendszerének javítása

Conference for PhD students, BUTE, Budapest, Hungary, 4 February 2011

Török Kitti, Bugyi Zsuzsanna, Hajas Lívia, Adonyi Zsanett, Tömösközi Sándor: Gliadin meghatározására alkalmas ELISA módszerek összehasonlítása, a validálás feltételrendszerének javítása