AF/03

ALLERRISK - Results

Development of an integrated strategy for controlling the allergen issue in the Belgian food and catering industry

DURATION OF THE PROJECT
15/12/2006 – 31/01/2011

BUDGET
799.231 €

KEYWORDS
Allergens, ELISA, PCR, mass spectrometry, food stuffs

CONTEXT

Food allergies represent an important health problem and the prevalence of allergic reactions shows an increasing trend over the last years. Currently, the only effective treatment for food allergy is avoidance of the allergen-containing food. The screening of food products to detect possible allergens is an essential part of a solid preventive policy at the level of the public authorities and the food industry. The food industry is actually confronted with a problem with regard to the validation of their production processes in order to assure absence of cross contamination in the production lines and also with a reliable quality control of the incoming materials. Due to the specific characteristics and the high level of variability among allergens, the development of functional methods to detect the most important allergens will allow public authorities to compose a new preventive policy in order to improve food safety.

OBJECTIVES

The principal goal of this study was to evaluate different methodologies for allergen detection in food and develop new, improved assays.
In this study we linked the results of the bio-analytical evaluations to the clinical impact for the allergic patient. Therefore, it was important that the bio-analytes used for the studies were clinically relevant, i.e. containing the relevant allergens and in a form being able to provoke an allergic response. Evaluation of different hazelnut protein isolation protocols highlighted the relevance of applying protease-inhibitors during the extraction procedure to preserve the proteins in their native conformation.
The investigated commercial ELISA's showed a lower analytical sensitivity than indicated by the manual. The hazelnut kits were able to detect 1 ppm of defatted hazelnut powder in food model systems, corresponding to about 2,6 ppm hazelnut.

The evaluated PCR assays for hazelnut detection showed a positive result for all replicates tested starting from 100 ppm defatted hazelnut flour in the food model systems. The lower sensitivity of the real-time PCR detection platform is probably related to the lower abundance of DNA as target analyte compared to the proteins targeted in ELISA, rather than being inhering to the PCR assay itself. The selected DNA isolation protocol yielded DNA of good quality with respect to the purity and integrity of the DNA. High purity has often a negative impact on the yield. Perhaps the sensitivity of the PCR platform could be increased by applying a DNA isolation protocol returning a higher yield. Our results also confirm that a higher sensitivity is generally associated with a lower specificity, as observed for the commercial ELISA assays for hazelnut and soy evaluated in this project.

Food processing simulating reactions had a severe impact on proteins. As a result of the Maillard reaction, increases in protein bound carbonyls, losses of available lysine residues and severe aggregation was observed. Due to oxidation, either lipid- or hypochlorous acid-induced, modification of essential amino acid was observed linked to an increase in protein bound carbonyls and severe protein aggregation. Further, partial peptic hydrolysis led to almost complete hydrolysis of the hazelnut proteins while the soybean proteins were more stable as evidenced by increases in the free amino groups and the non-protein nitrogen fraction. Despite these severe changes on protein level, several peptides derived from hazelnut and soybean proteins remained stable. Peptides derived from the Cor a 9 allergen from hazelnut and the Gly m 5 and Gly m 6 from soybean were shown to be the most stable as compared to other allergens. These stable peptides could serve as analytical targets for the development of new robust analytical approaches for detection of undeclared allergens in especially highly processed foods.

SSD SCIENCE FOR A SUSTAINABLE DEVELOPMENT

AGRO-FOOD

Further, both the ELISA and real-time PCR detection platform were susceptible to food processing and suffered from interference of the matrix. This was also evidenced by studying the impact of food processing simulation reactions on the detection of hazelnut and soy by commercial ELISA kits, where the observed impact on the detectability was highly dependent on the ELISA kit used. Based on the obtained results it cannot be concluded whether DNA is less susceptible to food processing than proteins, as is often stated. Only a semi-quantitative comparison could be made between both detection platforms, indicating that the impact of food processing was highly variable between the different assays from both types of detection methods.

The commercial ELISA's proved to fail detecting or correctly quantifying the (processed) proteins possibly because the antibodies used for their development are generated against native proteins and their reactivity towards the modified proteins is decreased because the specific epitopes are altered during food processing. Therefore, new ELISA assays were developed using antibodies against modified protein extracts. The newly developed ELISA's showed high specificity towards the modified hazelnut and soybean, with relatively low specificity towards the native ones and without any additional cross reactivity with other nuts or legumes.

The robustness of the developed ELISA's was assessed in in-house baked cookies spiked at different levels. Upon spiking of the blank cookies before the extraction, an extensively used approach for the assessment of the robustness of the ELISA, high recoveries were obtained for a wider range of spiking levels. However, upon spiking before baking, low recoveries were obtained probably due to decreased extractability of the antigen from the processed food. A comparison of the performance of the ELISA using antibodies against modified protein extract with a new ELISA against Kunitz trypsin inhibitor (reported stable protein) was made. This showed that using antibodies developed towards allergens modified through food processing simulating reactions is a better approach to be used in food allergen detection.

Target peptides specific of major soy and hazelnut allergens were chosen in the aim of developing a quantitative mass spectrometry-based method to detect trace amounts of allergens in food. For hazelnut, a method for the detection and quantification of 11S globulin-like protein (Cor a 9) in food has been developed. Semi-quantification of Cor a 9 was carried out in shop-bought chocolate and cookies and in home-made cookies. This is the first mass spectrometric method for the detection and semi quantification for Cor a 9. For soy, two calibration curves consisting of either uncooked or baked cookies spiked with increasing amounts of soybean protein extract were constructed. The limit of detection was determined for the baked cookies: 50 ppm soybean protein extract spiked. Two shop-bought samples were analysed: a type of biscuit and a cream dessert. The method worked well for both.

CONCLUSIONS

The most important conclusion of this research is that basophils of patients with documented severe hazelnut allergy can be used to demonstrate functional active hazelnut allergens in different matrices. Although this technique cannot be used on a routine base, it might be most valuable, to analyze food containing potentially clinically relevant traces of allergen which might not be demonstrated using "classical" techniques like ELISA or PCR.

At present, the available routine detection methods cannot be applied to assess the remaining allergenicity in food products. Routine assays can only give information on the presence of the food species or particular allergens. As explained above, allergenicity can only be tested with functional assays applying blood from allergic individuals, which makes these tests unfeasible as routine tests. However, it must be noted that the BAT has a very good sensitivity as compared to the other evaluated methods.

ALLERRISK - Results

Development of an integrated strategy for controlling the allergen issue in the Belgian food and catering industry

This study clarifies that both ELISA and PCR are in theory equally suited for allergen detection in food as they are both only to provide information on the presence of the concerning allergeninc ingredient. Even if an ELISA assay targets the particular allergenic proteins, it cannot serve as an allergy assessment tool, as it merely detects the protein but cannot determine the allergic potential. The assessment of the commercial analytical tools proves that the accuracy of the quantification/detection is undermined by food processing and matrix effects. Performing a risk assessment concerning the impact of food processing on the detection of food allergens by means of routine/commercial tests is complicated, as the results of this study have demonstrated that the impact of chemical modifications as induced by the processing is dependent on the assay used (detection) and moreover varies among sensitised individuals (allergenicity). With regard to studies investigating the impact of food processing it must also be emphasised that extraction of the analyte from the food matrix after processing remains an issue for the different detection platforms (ELISA, PCR, MS, BAT). In this context, MS based detection methods are promising as more harsh extraction procedures can be applied here, as this technique does not analyse intact proteins but detects peptides instead.

To conclude, this study has demonstrated that kit developers should be urged to perform a more extensive evaluation of the currently available routine tests, similarly as has been performed within this project. This information should support the different concerned actors to make an educated selection on the most appropriate test for the intended application..

CONTACT INFORMATION

Coordinators

Marc De Loose & Els Daeseleire
Institute for Agricultural and Fisheries Research (ILVO)
Technology and Food Unit (T&V)
Brusselsesteenweg 370
9090 Melle
Tel: +32 (0)9 272 28 42 (Marc De Loose)
Tel: +32 (0)9 272 30 32 (Els Daeseleire)
Tel: +32 (0)9 272 30 11 (Wim Reybroeck)
Fax:+32 (0)9 272 30 01
marc.deloose@ilvo.vlaanderen.be
els.daeseleire@ilvo.vlaanderen.be
wim.reybroeck@ilvo.vlaanderen.be
http://www.ilvo.vlaanderen.be/TenV/

Promoters

Bruno De Meulenaer
Universiteit Gent (UGent)
Research Group Food Chemistry and Human Nutrition
Coupure Links 653, B-9000 Gent
Tel: +32 (0)9 264 61 66
Fax: +32 (0)9 264 62 15
bruno.demeulenaer@ugent.be
http://www.foodscience.ugent.be/

Bart Devreese
Universiteit Gent (UGent)
Laboratory for Protein Biochemistry and Protein Engineering
K.L. Ledeganckstraat 35, B-9000 Gent
Tel: +32 (0)9 264 52 73
Fax: +32 (0)9 264 53 38
bart.devreese@ugent.be
http://www.eiwitbiochemie.ugent.be/

Edwin De Pauw &
Guy Maghuin-Rogister
Université de Liège (ULg)
Centre for Analysis of Residues in Traces
Institut de Chimie, Bat. B6c, B-4000 Liège
Tel: +32 (0)4 366 34 14 (Edwin De Pauw)
Tel: +32 (0)4 366 40 40 (Guy Maghuin-Rogister)
Fax:+32 (0)4 366 34 13 (Edwin De Pauw)
Fax:+32 (0)4 366 40 44 (Guy Maghuin-Rogister)
e.depauw@ulg.ac.be;
g.maghuin@ulg.ac.be
http://www.mslab.ulg.ac.be/cart/

Wim Stevens & Didier Ebo
Universiteit Antwerpen
Department for Immunology, Allergology and
Rheumatology, Campus Drie Eiken
Universiteitsplein 1, B-2610 Antwerpen
Tel: +32 (0)3 820 25 94 (Wim Stevens)
Tel: +32 (0)3 820 25 95 (Didier Ebo)
Fax: +32 (0)3 820 26 55
wim.stevens@ua.ac.be
didier.ebo@ua.ac.be, http://www.ua.ac.be

AGRO-FOOD

SSD SCIENCE FOR A SUSTAINABLE DEVELOPMENT

BELGIAN SCIENCE POLICY
Louizalaan 231 Avenue Louise • B-1050 Brussels
Tél. +32 (0)2 238 34 11 • Fax +32 (0)2 230 59 12 • www.belspo.be/ssd
Contact : Christine Mathieu