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METHODS FOR DETERMINATION OF THE PRESENCE OF ALLERGENS IN FOODS

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Abstract: Recent studies indicate that 2-4% of the world population is sensitive to the presence of allergens in foods. It is estimated that there are 5-8% allergic children in Europe.

This paper presents an overview of the analytical methods which are used in risk analysis and for making decisions in the management of foods that cause allergies in humans.

Depending on the purpose of analysis, a variety of analytical techniques can be used: Enzyme-linked immunosorbent assay (ELISA) is recommended for quantitative determination of the composition of ingredients and final products, Lateral flow test (LFD) can be used during routine control of the efficiency of purification and determination of the composition of final products, PCR (polymerase chain reaction)-ELISA and real-time PCR are used to confirm the ELISA results for samples with a low content of allergens or where it is not possible to apply other tests. Mass spectroscopy (MS) can be used as a method for confirming the results of the routine tests and in detecting of small amounts of the allergens. This paper gives an overview of these techniques by listing the advantages and disadvantages of routine food analysis.

Keywords: food allergens, methods of determination, ELISA, PCR, LFD.

Introduction

The primary goal of allergens analysis in food is to determine their characteristics and assess risks to human health (Burks et al., 2001). Numerous studies of adverse reaction to foods and their impact on human health were conducted. Kanny et al. (2001) and Zuberbier et al. (2004) found that nearly 4% of the population in Europe suffers from some form of food allergy. They believe that within the European Union the number of people who have such problems are between 10-20 million. In particular, children become more and more vulnerable and, unfortunately, they suffer more than adults (Sampson, 2005).

Even though there is no general agreement between scientific and medical communities on definition and division of the diseases produced by food intake, most experts accepted the division given by the European Academy of allergology and Clinical Immunology (EAACI) (Hayder et al., 2011). All reactions are classified as toxic and non-toxic (adverse reactions are divided into toxic and non-toxic reactions). Further, nontoxic reactions may be immune mediated or immune unmediated. In the first case we talk about food allergies, and in another we refer to food intolerance. Immunoglobulin (IgE) is the main antibody involved in the allergic reaction. Most of the literature on this issue deals with reactions related to the IgE mediation, although there is some research related to immunoglobulin IgG (Sampson, 1999). Food intoler-

ance reactions are commonly result of some certain enzymes reactions or some pharmaceuticals and chemical ingredients impact on individual (Kilburn, 2000). Furthermore, aversion to food is not included in the EAACI classification, for reactions do not repeat while taking food once again. Generally speaking, food aversion presents psychological intolerance and food avoidance (Ross et al., 2008).

Since proteins are the ones which cause allergic reactions, analytical techniques are focused on the identification and characterization of protein - allergens are very important tools for understanding the occurrence of food allergies and faster detection of allergenic ingredients in food products (Röder et al., 2009). Unfortunately, allergens are often found in traces, so their detection is quite difficult. This paper presents an overview of some of the methods that enable the identification and quantification of allergenic ingredients in food products.

Mechanism determination of adverse food reactions is essential for diseases symptoms and its treatment. It is also important for allergens elimination during food production and processing. In addition, the characteristics of allergens and reactions in which allergens are involved, were used for the development of some analytical methods for the identification and determination of specific allergens in food. The development of new and improvement of existing analytical techniques is one of the priorities in food safety management.

The aim of this paper is to present the methods that are available to researchers who deal with this issue, to show the advantages and limitations of individual methods and to find some methods that are most appropriate for some routine analysis.

Material and Methods

Laboratory of the Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" in Banja Luka is accredited as a reference laboratory for the analysis of food of animal origin. Taking into account more and more frequent requests for food analysis on presence of allergens by food producers, establishment of some special laboratory at the Institute on this issue became almost an imperative. In order to select a method for qualitative and quantitative determination of allergens in food products, it was necessary to do a comprehensive review of the analytical methods used in the world, and to identify directions for developing analytical methods that can be applied in routine work, so that the chosen method can be referred as reference in the process of laboratory accreditation. It should be pointed out, that colleagues from the University and the competent national authorities were involved in the work of the Institute for the collection, analysis and selection of the most acceptable method. In order to facilitate the work of other laboratories, part of the research study is presented in this paper.

Results and Discussion

Analytical methods can be used during all phases of management allergens and foods containing allergens. Different analyses were conducted in order to determine the following: the composition of foods containing allergens, determination of the content of allergens, determination of the amount of allergens in finishing products that are integrated with materials with allergens, condition and potential allergens after processing or storage products, validation of control measures undertaken to prevent cross-contamination, cleaning efficacy, effectiveness of barriers to prevent cross contamination and monitoring of suppliers.

In order to monitor the health status of patients and determine the susceptibility of individuals to adverse food reactions a large number of tests were developed (Zuidmeer et al., 2008; Ross et al., 2008; Savage et al., 2010; Jennifer et al., 2010).

In order to make a decision which analytical method will be purchased and used, it is of great importance to understand the nature of allergen molecules, limits of required detection sensitivity and specificity of the methods and procedures for sampling and extraction. Most of the techniques which are used in diagnostic assays or the identification of allergens are focused on the determination of protein components in the food.

Food that causes allergic reactions in susceptible individuals may be different. However, the list of common allergens is relatively short. Common causes of allergies include: cereals containing gluten, milk, eggs, peanuts, nuts, fish, shellfish, soy, shrimp, sesame, celery, SO₂ (Directive 2003/89 / EC, 2003; Directive 2007/68 / EC, 2007). In order to cause allergic reactions, food must contain substances that are immunogenic and should lead to allergic sensitization. As a result, IgE antibodies are formed. Most of the reported allergens are proteins, although some carbohydrates can bind IgE. Lipids do not cause allergic reactions (Blanco et al., 1999).

The functional properties of the protein and the binding ability of the antibody depends on the arrangement of amino acids in the protein chain, three-dimensional structure and the nature of the side groups of the protein molecule. Most of the allergens reported in the literature, are proteins which may be associated with a carbohydrate and their molecular weight was in the range of 10-100 kDa. Most of the proteins in food are immunogen and induce the formation of specific antibodies (mainly IgG). Only small number of proteins affects the production of IgE antibodies. They are considered as allergens. Protein molecules that induce immune responses typically have a size over 7000 daltons (Nagy et al., 2002; Janssen, 2006; Skripak et al., 2007).

Methods for determination of allergens are carried out at several levels: physico-chemical and biochemical methods for *in vitro* measurement of specific antibodies in food, *in vitro* determination of amounts of general and specific IgE antibodies; animal testing responses to allergens and clinical studies on patient response using different tests on the skin, respiratory, digestive organs, etc. (Beyer et al., 2002; Jarvinen et al., 2007; Hirsch et al., 2003; Jedrychowski et al., 2010).

Method, location and frequency of taking the samples should be based on risk assessment. Samples should be taken with a especially clean equipment, to be placed in clean containers and, if necessary, with cooling and short delivery to the laboratory. Type of sample depends on the specifics of the activities in the production environment and activity that is under monitoring process. Prior to assay, allergens substances must be extracted from food. As techniques for the extraction of allergens, following techniques are commonly cited in the literature (Van Ree et al., 2006):

- Separation on a gel such as SDS PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) (SDS-PAGE) followed by Western blotting and immuno-labelling,
- Separation by chromatography (often High Performance Liquid Chromatography) followed by ELISA.

In different cases, different methods are used to analyze allergen. From classic physico-chemical and biochemical methods for protein testing following methods can be used: Physicochemical methods, Kjeldahl nitrogen assay, Nephelometry, Colorimetry, Chromatography (SEC, ion-exchange chromatography, affinity chromatography, HPLC, FPLC), Electrophoresis (SDS PAGE, capillary electrophoresis, 2D - electrophoresis), Spectrophotometry, Mass spectrometry and PCR (DNA specific for allergen). Immunological methods, as follows: Counter electrophoresis, immunoblotting, Immunodiffusion, enzyme - linked immunosorbent assay (ELISA), enzyme-linked immunospot assay (ELISPOT), Radioimmunoassay.

The literature describes numerous Immunochemical methods for the determination of allergens, including: dipsticks, biosensors and immunoblot and immunoaffinity columns, Enzyme-linked immuno-

sorbent assays (ELISA). They all have important applications in clinical diagnostics, and in the last ten years their application for assessment of allergen in food has grown (Yeung, 2006). Kits which are offered commercially, are distinguished by detection limit of allergens (for example, the detection limit for peanut residues ranges from 0.5 ppm to 5 ppm). Two-dimensional electrophoresis allows the separation of proteins with similar molecular weights. Immunoblotting is also used in different ways for testing of food allergens. It is most commonly used in qualitative studies, or in identifying molecules that bind allergens (IgE). This technique has been successfully used for the detection of allergens in food products (for example, traces of hazelnuts and almonds in chocolate) (Scheibe et al., 2001).

Several papers have been published on the application of PCR assays for detection and quantification of residues of foods containing allergens (hazelnut, peanut, celery, wheat, soy, etc.) (Holzhauser et al., 2006). Similar to ELISA, PCR's sensitivity limit for the majority of residues ranging up to 10 mg / kg. Methods based on DNA analysis (PCR) can be employed in the analysis of hidden allergens in finishing food products and can be supplemented by ELISA. ELISA method may be used in the detection of eggs and milk as food allergens, while PCR assays should be used in the identification between closely related products (walnut, hazelnut, almond). Commercially available PCR - ELISA and real-time PCR assays for the detection of hidden allergens in the finished product (Poms et al., 2004).

From each test / analytical method, sensitivity, selectivity, specificity and reproducibility is required and to be confirmed for each type of food being analyzed.

Laboratories where analyses for allergens presence are carried out, should be adequately equipped and should have all the equipment necessary to perform this type of analysis, and employ staff who are well trained for these jobs. The type of food and its condition are also important for the accuracy of the results. Products of different physical state and with different degrees of homogeneity require a different approach and attention during preparation for analysis. The material which is analyzed can contain some ingredients which have ability to mask allergens. Furthermore, investigated material can also contain some substances that are not suitable for certain types of tests and analyses. In such cases it is possible to get a false result (less or greater than the actual value of the allergens content).

Conclusions

- An intensive application of some novel techniques for qualitative and quantitative determination of allergens can be expected in the future.
- Methods which deal with proteins reaction will have advantage comparing to those based on DNA detection.
- For faster development of sensitive and specific analytical method for the quantitative determination of allergens in finishing food products, issue of the lack of appropriate standard reference materials should be solved.
- Automated tests and time-less consumed methods will be prior to long term laboratory analysis.
- Automated test will reduce the need for manual manipulation, work of highly skilled workers, will impact on the acceleration of analysis and reduce errors caused by human labor. In this regard, emphasis on the application of Dipstick technology and biosensors will be significant.

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