

Introducing allergists to food chemistry

A report of common analytical methods for the identification and characterization of food allergens is presented. The different electrophoretic and immunologic methods describe the present analytical tools to characterize allergens. With respect to the lack of knowledge on the structure and the properties of food allergens, additional chemical techniques such as chromatography, NMR and MALDI-TOF are of particular importance.

H. Steinhart, M. Wigotzki, K. Zunker

University of Hamburg, Institute of Biochemistry and
Food Chemistry, Hamburg, Germany

Key words: allergens; identification; characterization;
analytical methods.

Prof. Dr H. Steinhart
University of Hamburg
Institute of Biochemistry and Food Chemistry
Grindelallee 117
D-20146 Hamburg
Germany
Tel: +49-40-428384356/7
Fax: +49-40-428384342

The connection between food chemistry and allergology is obvious, considering the structure of food allergens, which are proteins or glycoproteins. For their isolation from the food matrix and their chemical characterization the analytical knowledge of food chemists is required.

The isolation of proteins and glycoproteins in a native form is the prerequisite for the extraction from foodstuffs. A suitable method for vegetable food is the low temperature extraction developed by Clements (1) and modified by Vieths et al. (2). The foodstuffs are homogenized with cold acetone at -40°C in order to separate the phenolic ingredients, which can cause a loss of allergenic activity by enzymatic reactions. The precipitated proteins are washed, lyophilized and extracted by means of phosphate buffered saline. The contents of the obtained protein extracts are measured by the method of Bradford (3).

The separation of the different proteins from food is carried out by applying chromatographic and electrophoretic methods. The electrophoretic methods especially include sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric

focusing (IEF). While in the case of SDS-PAGE the separation of the proteins is carried out only according to their molecular weight, IEF is used to separate proteins and peptides by their isoelectric points. 2D-electrophoresis results from the combination of IEF and SDS-PAGE. By using 2D-electrophoresis a high resolution of complex protein mixtures and the separation of isoproteins can be achieved (4). The nonspecific detection of electrophoretically separated proteins in polyacrylamide gels is generally carried out by means of silver staining (5) or Coomassie brilliant blue staining (6). Capillary electrophoresis (CE) represents a further possibility for the separation of proteins and peptides. The increased development of different separation techniques in recent years shows a strong demand for the conjunction of CE and further characterization methods (7).

Apart from electrophoretic techniques the use of immunologic methods is of great importance with regard to the identification and characterization of allergens. The specific determination of food allergens can be carried out by immunoblotting and enzyme linked immunosorbent assays (ELISA).

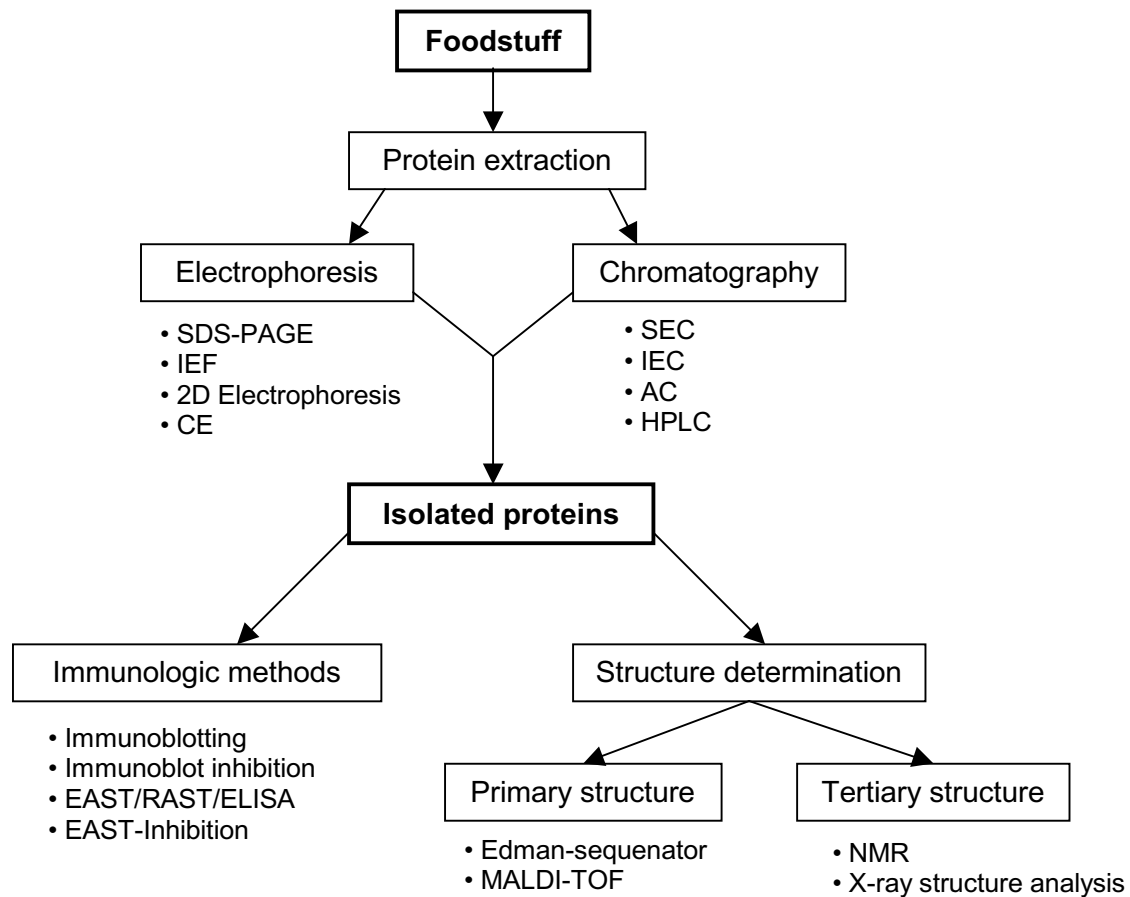


Figure 1. Analytical methods for the determination of food allergens (abbreviations are explained in the text).

Immunoblotting means the combination of semidry-blotting of electrophoretically separated proteins or peptides (8) followed by an immuno-detection with specific antibodies (EAST=enzyme allergosorbent test; RAST=radio allergosorbent test) (9). For quantification of allergens, different ELISA-techniques are used.

Besides the identification of the allergens by electrophoretic methods, the characterization of epitope structures by chromatography is important. The determination of the sequence or the configuration of the epitopes is only possible with chemical analysis - immunologic methods are impracticable for this purpose. Important chromatographic methods are size exclusion chromatography (SEC), ion exchange chromatography (IEC), and high performance liquid chromatography (HPLC). These chromatographic separations are based on different chemical and physical properties of the proteins,

such as molecular weight, charge or pH. Affinity chromatography (AC) is also a widely used chromatographic analysis method, although it is based on immunological antigen-antibody reactions.

The chromatographically isolated and purified protein extracts can be used to characterize their chemical structure by means of nuclear magnetic resonance spectroscopy (NMR) and X-ray structure analysis. The determination of the primary structure is carried out with an Edman sequenator. In this case, amino acids react with phenylisothiocyanate to phenylthiohydantoin derivatives, which can be identified by applying HPLC. A maximum of 20 cycles is possible (10). The molecular weights of peptides and proteins are determined by mass spectrometry. Matrix assisted laser desorption/ionization-time-of-flight (MALDI-TOF) represents a gentle method for the determination of large molecules such as native proteins.

References

1. CLEMENTS RL. Fruit proteins: extraction and electrophoresis. *Anal Biochem* 1965;**13**:390–401.
2. VIETHS S, SCHÖNING B, BROCKMANN S, AULEPP H. Untersuchungen zur Allergie gegen Lebensmittel pflanzlicher Herkunft: Herstellung und Charakterisierung von Obst- und Gemüseextrakten für serologische Untersuchungen. *Dtsch Lebensm Rundsch* 1992;**88**:239–243, 273–279.
3. BRADFORD MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–254.
4. GÖRG A, POSTEL W, GÜNTHER S. The current status of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1988;**9**: 531–546.
5. HEUKESHOVEN J, DERNICK R. In: RADOLA BJ, editor. *Neue Ergebnisse zum Mechanismus der Silberfärbung*. Munich: Elektrophores-Forum, 1986:22–27.
6. FLING PS, GREGERSON DS. Peptide and protein determination by using a high molarity tris buffer system without urea. *Anal Biochem* 1986;**155**:83–88.
7. BESLER M, STEINHART H, PASCHKE A. Allergenicity of hen's egg-white proteins: IgE-binding of native and deglycosylated ovomucoid. *Food Agric Immunol* 1997;**9**:277–288.
8. KYHSE-ANDERSEN J. Electrophoretic transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Meth* 1984;**10**: 203–209.
9. VIETHS S, SCHÖNING B, BALTES W. Allergy to fruits and vegetables in pollen-sensitive patients: allergen characterization by IgE immunoblotting and peroxidase staining. *Food Agric Immunol* 1992;**4**:181–197.
10. EDMAN P, BEGG G. A protein sequenator. *Eur J Biochem* 1967;**1**:80–91.