Dynamics of Allergen Degradation in Food

M. KOVAC, B. KRKOSKOVA, H. STRAZNICKA, M. SIMKOVA

Food Research Institute, Priemyselná 4, P.O.Box 25, 82006 Bratislava, Slovakia.

Summary

In an attempt to develop objective methods for the detection of potential allergens in food obtained from legumes, this study focused on the procedures of gel chromatography or electrophoresis used in monitoring protein fractions and their derivatives after technological treatment by enzymatic hydrolysis to decrease allergic reactions.

Introduction

Food allergens can be defined as those substances that initiate or produce immunological reactions of food allergy. Food allergy is most frequent in infants and most serious in adults. Between 0.5% and 10% of normal infants will develop some kind of food allergy (Cordle, 1994). In Slovakia, food allergies constitute around 42% of the total allergies developed. The most frequent food-induced allergies are those to alfa-lactalbumin, beta-lactoglobulin and ovalbumin. Allergens are usually naturally occurring proteins found in food. Allergenic proteins are typically 10/70 kDa in molecular mass and often glycosylated. The immunogenicity of proteins is generally related to their amino acid sequence and three-dimensional structure. Legumes are common allergenic foods, and soybeans contain multiple allergens. The allergens in green peas have been characterized to some extent (Taylor, 1992). Since food allergens are proteins, protein hydrolysis would be likely to decrease the allergenicity of foods (Cordle, 1994). The detection of food allergens or allergenic foods can be important for evaluation of the allergenicity of specific foods or assessment of the effect of processing on the allergenicity of specific foods. Allergens can be identified using traditional in vitro assays such as RAST inhibition or ELISA inhibition assays. Both assays are based on the competitive binding of IgE antibodies in patient serum to the test sample, as compared with the solid-phase allergen. Tests of increasing inhibitor concentrations can be used to measure unknown allergen quantities in assay
samples (Hefle, 1995). The present study considered the utilization of enzymatic hydrolysis in reducing the allergenicity of some legume proteins.

Materials and methods

Samples of dry seeds of peas (*Pisum sativum* L., var. Janus, Olivin, Sirius) were examined, as well as protein concentrates and protein fractions prepared in the laboratory. Samples of pulses were used as defatted grits. Technologically treated samples were represented by pea protein hydrolysates.

**Enzymatic hydrolysis.** Pea protein extracts were hydrolyzed by various food-grade enzymes (Alcalase 2.4L, Neutrase 1.5 MG, Novo Industri A/S, and Pepsinum, Léèiva Prague). Hydrolysis was carried out under standard conditions for each enzyme.

**Gel chromatography.** Legume proteins were extracted from defatted milled samples and fractionated, as described previously (Krkosková and Paulen, 1995). Samples of vicilin, legumin, the albumin fraction and the whole extract were loaded on a SephacryleS-200 HR column, which was then eluted with borate buffer, pH 8.5, at the rate of 1 ml/min. Fractions (5 ml) were collected and analyzed by electrophoresis.

**Electrophoretic analyses.** Protein fractions were analyzed by SDS-PAGE according to the method of Laemmli, which was slightly modified to be used with the Mighty Small Electrophoresis Unit (Hoefer Pharmacia Biotech.). Gels were 0.1 mm thick, consisting of a 1-2 cm stacking gel and 6-8 cm running gel. The final electrophoretic conditions were: concentration of running gel 12.7% T, 2.6% C, buffer 0.45 M TRIS-HCI, pH 8.8; and concentration of stacking gel 4.1% T, 3.1% C, buffer 0.15 M TRIS-HCI, pH 6.8.

Samples in the sample buffer were boiled for 5 min before being loaded. Electrophoresis was carried out for 4 h at 70 V, 13 A; 130 V, 22 A (stacking and running gel) respectively. Gels were stained for 2 h with 0.1% Coomasie brilliant blue R 250, methanol:water:acetic acid (4.5:4.5:1). Destaining solvent was from ethanol:glycerol:water (2:1:7).

Molecular weight standards (Pharmacia) were used to estimate protein subunit molecular weights (Hames and Rickwood, 1983).

Results and discussion

**Gel chromatography.** Four fractions were obtained by gel filtration of pea proteins. The first eluted fraction contained only traces of protein, nucleic acids, sugar and other substances. The globulins were eluted in two peaks for the 11S