

Protein modification by fermentation: effect of fermentation on the potential allergenicity of pea

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The effect of fermentation on components of potential significance for the allergenicity of pea was analyzed. Pea flour was fermented with three lactic acid bacteria, *Pediococcus pentosaceus*, *Lactococcus raffinolactis*, and *Lactobacillus plantarum*, and two fungi, *Rhizopus microsporus*, var. *oligosporus* and *Geotrichum candidum*. Residual antigenicity against anti-pea antibodies was reduced to 10% by the three lactic acid bacteria and *R. microsporus*. Reactions to anti-pea profilin and anti-Bet v 1 were still detectable after fermentation. The contents of lectin and pea protease inhibitor were not reduced by the microorganisms.

Key words: antigenicity; Bet v 1; digestion *in vitro*; ELISA; fermentation; lectin; pea (*Pisum sativum*); profilin.

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The European pea crop could be utilized as a source of vegetable protein instead of soybeans, which are currently used as the major vegetable protein in industrially produced food. But pea, like other legumes, contains various antipathogenic factors such as phytate, protease inhibitors, lectin, saponin, and indigestible oligosaccharides of the raffinose series. In Oriental foods, biofermentation by lactic acid bacteria, fungi, and yeasts is traditional. Our objective was to select lactic acid bacteria and fungal strains suitable for biofermentation of peas. Selection criteria were based on the ability of strains to decrease the antinutritional components such as phytic acid, their ability to ferment α -galactoligosaccharides, and their antimicrobial activity (1, 2).

The present study aimed to investigate the effect of fermentation on components of potential importance for the allergenicity of pea. Available information about the allergenicity of pea is limited compared to the information about other legumes, especially soybean. Although there is extensive immunochemical cross-reactivity among legumes, clinical cross-reactivity is very rare (3). No specific proteins have been identified as major allergens in pea. Malley et al. (4) found that the albumin

fraction represents the allergenic fraction of pea, whereas Bernhisel-Broadbent et al. (5) demonstrated IgE binding to apparently all fractions. This is in accordance with the analyses of Burks et al. (6) on the allergenicity of soybean protein. To evaluate the allergenic potential of fermented pea flour compared to unprocessed pea flour, we determined the residual antigenic potential of the total protein mixture. Specific analyses were made for the potentially cross-reacting inhalation allergens, Bet v 1 (7) and profilin (8). Investigations of molecular size distribution and accessibility to *in vitro* digestion provided information on proteins or large fragments that may resist degradation in the digestive tract and cross the mucosa with some immunogenic structure left.

Protease inhibitors were determined because they may affect the digestibility of allergens. In addition to the IgE-binding allergens, allergenicity may depend on factors promoting allergenicity of accompanying components. Little is known about such factors, but lectins may be of importance because of their ability to bind to carbohydrate in the enterocytes in the gut and to stimulate immunocompetent cells in the mucosa (9).

Material and methods

Fermentations

Five fermented samples were analyzed, three of them fermented with lactic acid bacteria and two with fungi. All fermentations were carried out for 48 h with irradiated (10 Gy), finely milled pea flour. The references were unfermented, irradiated pea flour incubated in the same conditions. The moisture content was 70% for the bacteria *Pediococcus pentosaceus*, *Lactococcus raffinolactis*, and *Lactobacillus plantarum*. The moisture content was 40% for the fungi *Rhizopus microsporus*, var. *oligosporus* (used for tempeh fermentation) and *Geotrichum candidum* (used in cheese making).

Antigens and antisera

Pea profilin was isolated by a method modified from that of Vidale et al. (10) and used for immunization of rabbits. Rabbit antibodies against Bet v 1 were a kind gift from ALK, Hørsholm, Denmark. Extraction of pea proteins was performed overnight with 0.1 M NH_4HCO_3 , and the extract was used for immunization of rabbits.

Analyses

SDS-PAGE was run on Tris/tricine gels 10–20% (Novex) and immunoblotting performed according to Ramlau (11). Antigenicity was determined by sandwich ELISA: microtiter plates were coated with rabbit antibodies against native pea proteins, incubated with twofold dilutions of extracts of fermentation mixtures, and detected with biotinylated antibodies followed by horseradish peroxidase-coupled streptavidin. Color was developed with tetramethylbenzidine and read at 450 nm. Titers were determined as the dilution giving an absorbance of 0.2. Binding of anti-Bet v 1 and anti-pea profilin was detected in microtiter plates coated with extracts. The negative controls for antigen and antibodies were casein and rabbit anti-mouse immunoglobulins, respectively. Proteinase inhibitor and lectin were determined by ELISAs based on monoclonal antibodies (12). *In vitro* digestion included adjustment with HCl to pH 2–3 and digestion with bovine pepsin (Sigma) for 1 h, followed by adjustment to pH 8 with $\text{NH}_3/\text{NH}_4\text{HCO}_3$, and digestion with pancreatin (Sigma) in the presence of taurocholate for 1 h. The protein concentration of extracts was determined by amino-acid analysis (13).

Results

The effect of fermentation on pea proteins was evaluated by SDS-PAGE and immunoblotting. Fermentation with the three lactic acid bacteria *P. pentosaceus*, *L. raffinolactis*, and *L. plantarum* resulted in similar patterns of the Coomassie brilliant blue-stained gels, not very different from the extract of the unfermented flour, except for the disappearance of some high-molecular-weight bands. *R. microsporus* gave a marked change of the pattern in the high- as well as the low-molecular-weight region. The corresponding patterns from immunoblottings with antiserum against an extract of native pea proteins were different from the patterns of the flour, and the staining was weaker. Fermentation with *G. candidum* gave no detectable change in the patterns. ELISA showed that fermentation with lactic acid bacteria and *R. microsporus* reduced the antigenicity to 10% of the antigenicity of the unfermented pea flour. *G. candidum* had no effect in accordance with the SDS-PAGE. Binding of anti-Bet v 1 and anti-pea profilin was detected in extracts of unfermented as well as fermented pea flour. The content of lectin and protease inhibitor did not change because of fermentation. *In vitro* digestion was tested after fermentation with *P. pentosaceus* and *R. microsporus* and compared by analysis of residual antigenicity to *in vitro* digestion of unfermented flour. Apparently, fermentation with *R. microsporus* increased the digestibility.

Discussion

The frequency of reported allergenicity to pea is lower than that of soybean and peanut (14). This may reflect a lower consumption of pea, but it may also reflect a lower allergenicity of pea. It is worth mentioning in that connection that part of the consumed peas are eaten raw. There is a long tradition of the fermentation of soybeans, and the effect of fermentation on soybean allergens has been investigated. Tsuji et al. (15) found that a major allergen *Gly m Bd 30K* was not detectable in three fermented products, miso, shoyu, and natto, whereas Herian et al. (16) found considerable residual RAST inhibition in the fermented products miso, tempeh, and tofu. In accordance with this, the results from the present study show that detectable amounts of antibody-binding material from all analyzed components remain after fermentation. All fermentations in the present investigation were performed without heat treatment of the pea flour. Denaturation of the pea proteins may facilitate the digestibility and increase the effect of fermentation on the allergens. Evaluation of the

allergenic potential of a product should include analyses of not only proteins, as other factors in the matrix may promote allergenicity. For example, for pea products, saponin may influence the absorption (17). T-cell proliferation assays with or without preceding feeding trial may reveal important information about the allergenic potential of a food product.

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