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Review

Aspects of food processing and its effect on allergen structure

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The article summarizes current physical and chemical methods in food processing as storage, preparation, separation, isolation or purification and thermal application on the one hand as well as enzymatic treatment on the other and their impact on the properties of food proteins. Novel methods of food processing like high pressure, electric field application or irradiation and their impact on food allergens are presented. The EU project REDALL (Reduced Allergenicity of Processed Foods, Containing Animal Allergens: QLK1-CT-2002-02687) showed that by a combination of enzyme and heat treatment the allergic potential of hen's egg decreased about 100 fold. Clinical reactions do not appear anymore. An AiF-FV 12024 N project worked with fruits like mango, lychee and apple. Processed mango and lychee had no change in allergenic potential during heating while *e.g.* canning. Apple almost lost its allergenic potential after pasteurization in juice production.

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1 Introduction

Beside from being stored, food is processed by a large number of diverse physical and chemical procedures used singly or in combination, depending on the starting material and the desired outcome. Some food processing methods are mechanical in nature *i. e.*, separation, isolation and/or purification while others are thermal like pasteurization, cooking and roasting including diverse chemical reactions as Maillard or there are biochemical methods like enzymatic treatment of food. Genetic engineering is a relatively new approach, which change the proteome both in, unprocessed and processed food *e.g.* when using enzymes of GMOs. Other less common or novel processes include high pressure, electric field treatment or irradiation. This paper reviews current knowledge of how food processing modifies the allergenic potential of food.

2 Food processing – change of proteome

Post harvest storage of food crops influences their properties and the outcome of food preparation techniques. For example, the proteome of fruits and vegetables can change during storage [1]. This can result in higher allergenicity, as shown for apples [2], but not for mangoes, for which there was no change in IgE binding potency during storage [3]. In general, when allergenicity changed during storage or ripening, storage conditions, including the gas composition of the ambient air during storage, were found to modulate the velocity of the storage-induced changes [4].

Protein concentration and content varies in different parts of a fruit or vegetable; thus, removal of one or more of plant parts can influence the allergenicity of the product. For example, peeling of peaches prior to preparation of peach juice is reported to reduce the allergenic potency of the juice product [5].

Mechanical processes such as stirring or homogenization might also influence the properties of food proteins. For example, such manipulation might cause surface denaturation of food proteins; however, a significant influence on allergenic potency has not been demonstrated [6].

Physical manipulations such as separation, isolation or purification procedures can alter food allergenicity. Examples are removal of starch from potatoes or wheat, and production of butter from raw milk; these processes nearly completely remove the proteinogenic (*i. e.*, allergenic) fraction of the unprocessed food. Purification by ultrafiltration is very effective for reducing allergenic proteins. Alternatively, hypoallergenic products, such as infant formula, have been prepared by treating milk with proteolytic

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enzymes; this process only leave a small amount of intact protein in the resulting product, and these residual intact allergenic proteins can be removed by ultrafiltration [7].

Thermal food preparation methods include baking, cooking, roasting, grilling, drying, pasteurization and sterilization. Heat-induced denaturation of proteins and/or reaction of food proteins with the food matrix could reduce allergenic potency of the food product. Protein denaturation reduces allergenicity by altering protein conformation, which destroys IgE-reactive conformational epitopes. Fiocchi and coworkers [8] showed that thermal treatment reduced the allergenicity of beef and purified bovine allergens. However, some allergens may be thermostable like the major peanut allergen Ara h 1, such that the IgE-reactive conformational epitopes are resistant to heat-induced denaturation, as shown by Koppleman and coworkers [9] for roasting peanuts. In some cases, heat-treatment causes protein denaturation, followed by spontaneous renaturation during cooling, as reported for the potato allergen patatin 8Sol t 1) [10], which also appear to form aggregates with other potato proteins during thermal processing. Food proteins also undergo chemical interactions with other food components, such as amino acids with sugars. One example is the Maillard reaction, which leads to brown coloration during heating or storage. This reaction develops aromatic compounds that modify the sensory aspects of cooked food, but it may also influence food allergenicity [11]. In cooked peanuts, products of the Maillard reaction are associated with IgE binding, which is less prevalent in untreated peanuts [12]. In milk, the reaction of beta-lactoglobulin with lactose also increases allergenicity [13]. Oxidative products, but not Maillard products, increase the allergenicity of pecans [14].

Biochemical food processing methods often involve use of enzymes such as proteases, oxidases or transglutaminases. Wigotzki and coworkers [15] showed that treatment with trypsin or elastase decreased the allergenicity of hazelnuts. Similarly, treatment of rice with actinase [16, 17], soybeans with proteases [18] and wheat with bromelain decreased the allergenicity of these foods [19, 20]. Although enzyme-mediated proteolysis did not destroy IgE-reactive epitopes in peanut or peach [21, 5], transglutaminase-treatment of casein [22] or wheat proteins [23] decreased allergenicity.

Genetic engineering has been used *e.g.* to generate novel variants of rice, soybean, peanut and apple which have lower allergenic potential [24–27] (EU project QLK1-2000-1394 SAFE Plant food allergies: Field to table strategies for reducing their incidence in Europe (http://www.akh-wien.ac.at/safe/). Unfortunately, this method is not very well accepted by consumers and increases food cost. Thus, it is unlikely that genetically engineered hypoallergenic foods will become commercially available products in the near future.

Kato and coworkers [28] and Jankiewicz and coworkers [29] investigated novel methods to decrease allergenicity. These included high pressure (100–400 MPa) treatment of rice [30] which reduced allergenicity. However, high pressure treatment of celery (600 MPa; [31]), as well as treatment with a pulsed electric field (10 kV, 50 Hz) or gamma-irradiation were reported to be ineffective in reducing allergenicity of celery [28].

The EU project REDALL (Reduced Allergenicity of Processed Foods, Containing Animal Allergens: QLK1-CT-2002-02687) (2003-2006) (www.chemie.uni-hamburg.de/lc/redall) is a collaborative effort among 13 participating groups from six countries, whose goal was to find procedures that reduce the allergenicity of milk, egg and meat products. REDALL was an interdisciplinary project involving analytical and food chemists, clinicians, consumer research scientists and food technologists. These scientists characterized unprocessed, processed food, and samples taken at intermediate steps during food processing. Standard skin prick tests were performed on volunteer patients as well as double-blind placebo-controlled food challenge tests; in addition, more sensitive methods were developed for chemical and clinical analytics. A consumer research institute also administered surveys to determine the prevalence of food allergy and severe allergic reactions to food in 10 European countries. Concerning egg and egg containing food products first results of reduced allergenicity were achieved. By a combination of enzyme and heat treatment the allergenic potential decreased about 100 fold [30, 31]. Egg allergic patients did not show reactions in skin prick test and double blind placebo controlled food challenge (unpublished data).

The Food Chemistry Section of the University of Hamburg and the Food Technology Section of the University of Hohenheim were sponsored by the FEI-Project (AiF-FV 12024 N), which conducted 'Studies on alterations of the allergenicity of fruits and vegetables during technological processing.' The FEI-Project focused on four fruits and vegetables (apple, mango, lychee and potato) and their products. The Food Technology Section investigated commercial procedures, while the Food Chemistry Section performed immunological characterization of purified food proteins and enzyme allergosorbent tests on raw, intermediate and final food products. Apple puree and decanted and pressed apple juices were tested. The intermediate products showed no loss of allergenic potency, and storage of mash for several hours or addition of ascorbic acid had little influence on its allergenicity. In contrast, pasteurization reduced IgE binding potency (unpublished data). Mango allergens were found to be heat-stable during production of mango nectar, and enzymes (polygalacturonase and pectin lyase) used to increase the yield of nectar did not reduce allergenicity at any stage of production of mango nectar [32]. During production of canned lychee fruit, allergenicity did not decrease with longer treatment at high temperature. Thus, it appears that lychee fruit allergens are heat-stable, despite the fact that low molecular weight IgE binding proteins

(20–35 kDa) were eliminated by heat treatment [33]. Lastly, potato flakes were produced using ascorbylpalmitate and monoglyceride. IgE binding proteins were reduced (but not eliminated) during processing, but a new IgE binding protein ∼50 kDa not further characterized also appeared. Ascorbylpalmitate and monoglyceride had no influence on allergenicity of the product [34].

3 Conclusions

Thermal and biochemical food processing and genetic engineering of novel plant variants have greater potential to alter food proteins than mechanical manipulation of food. Therefore, these methods have greater potential to reduce food allergenicity. However, in some cases, these methods may fail to reduce allergenic potential, may increase allergenic potential or may reveal 'neo'-epitopes which were masked in the native protein, but which become accessible and/or reactive after denaturation/renaturation of the protein. A great deal of current research is devoted to understanding precisely how traditional food processing methods influence food allergenicity. In addition, new methods for producing hypoallergenic foods or for preventing allergic responses to food are being explored. It remains important to preserve food quality and food identity while altering food protein to reduce food allergenicity.

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4 References

[1] Sell, M., Steinhart, H., Paschke, A., Influence of maturation on the alteration of allergenicty of green pea (*Pisum sativum* L.), *J. Agric. Food Chem.* 2005, *53*, 1717–1722.

[2] Vieths, S., Schöning, B., Jankiewicz, A., Occurrence of IgE binding allergens during ripening of apple fruits, *Food Agric. Immunol.* 1993, *5*, 93–105.

[3] Paschke, A., Kinder, H., Zunker, K., Wigotzki, M. *et al.*, Characterisation of allergens in mango fruit and ripening dependence of the allergenic potency, *Food Agric. Immunol.* 2001, *13*, 51–61.

[4] Li-Shan, H., Moos, M., Yuan, L., Characterization of apple 18 and 31 kd allergens by microsequencing and evaluation of their content during storage and ripening, *J. Allergy Clin. Immunol.* 1995, *96*, 960–970.

[5] Brenna, O., Pompei, C., Ortolani, C., Pravettoni, V. *et al.*, Technological processes to decrease the allergenicity of peach juice and nectar, *J. Agric. Food Chem.* 2000, *48*, 493–497.

[6] Paschke, A., Besler, M., Stability of bovine allergens during food processing, *Ann. Allergy, Asthma Immunol.* 2002, *89*, 16–20.

[7] Van Beresteijn, E. C. H., Peeters, R. A., Kaper, J., Meijer, R. J. G. *et al.*, Molecular mass distribution, immunological properties and nutritive value of whey protein hydrolysates, *J. Food. Sci.* 1994, *57*, 619–625.

[8] Fiocchi, A., Restani, P., Riva, E., Restelli, A. R. *et al.*, Meat allergy II – effects of food processing and enzymatic digestion on the allergenicity of bovine and ovine meats, *J. Am. Coll. Nutr.* 1995, *14*, 245–250.

[9] Koppleman, S. J., Bruijnzeel-Koomen, C. A., Hessing, M., De Jongh, H. H., Heat-induced conformational changes of Ara h 1, a major peanut allergen, do not effect its allergenic properties, *J. Biol. Chem.* 1999, *274*, 4770–4777.

[10] Koppleman, S. J., van Koningsveld, G. A., Knulst, A., Gruppen, H. *et al.*, Effect of heat-induced aggregation on the IgE binding patatin (Sol t 1) is dominated by other potato proteins, *J. Agric. Food Chem.* 2002, *50*, 1562–1568.

[11] Davis, P. J., Smales, C. M., James, D. C., How can thermal processing modify the antigenicity of proteins?, *Allergy* 2001, *56*, 56–60.

[12] Beyer, K., Morrow, E., Li, X.-M., Bardina, L. *et al.*, Effects of cooking methods on peanut allergenicity, *J. Allergy Clin. Immunol.* 2001, *107*, 1077–1081.

[13] Bleumink, E., Berrens, L., Synthetic approaches to the biological activity of beta-lactoglobulin in human allergy to cow's milk, *Nature* 1996, *212*, 514–543.

[14] Berrens, L., Neoallergens in heated pecan nut. Products of Maillard type degradation?, *Allergy* 1996, *51*, 277–278.

[15] Wigotzki, M., Schubert, S., Steinhart, H., Paschke, A., Effects of in vitro digestion on the IgE binding activity of proteins from hazelnut (*Corylus avellana*), Internet symposium on *Food, Allergens* 2000, *2*, 1–8.

[16] Watanabe, M., Miyakawa, J., Ikezawa, Z., Suzuki, Y. *et al.*, Production of hypoallergenic rice by enzymatic decomposition of constituent proteins, *J. Food Sci.* 1990a, *55*, 781–783.

[17] Watanabe, M., Yoshizawa, T., Miyakawa, J., Ikezawa, Z. *et al.*, Quality improvement and evaluation of hypoallergenic rice grains, *J. Food Sci.* 1990b, *55*, 1105–1107.

[18] Yamanishi, R., Tsuji, H., Bando, N., Yamada, Y. *et al.*, Reduction of allergenicity of soybean by treatment with proteases, *J. Nutr. Sci. Vitaminol.* 1996, *42*, 581–587.

[19] Tanabe, S., Arai, S., Yanagihara, Y., Takahashi, K. *et al.*, A major wheat allergen has a Gln-Gln-Gln-ProPro motif identified as an IgE binding epitope, *Biochem. Biophys. Res. Commun.* 1996, *219*, 290–293.

[20] Watanabe, M., Tanabe, S., Suzuki, T., Ikezawa, Z. *et al.*, Primary structure of an allergenic peptide occurring in the chymotryptic hydrolysate of gluten, *Biosci. Biotechnol. Biochem.* 1995, *59*, 1596–1597.

[21] Maleki, S. J., Kopper, R. A., Shin, D. S., Park, C.–W. *et al.*, Structure of the major peanut allergen Ara h 1 may protect IgE binding epitopes from degradation, *J. Immunol.* 2000b, *164*, 5844–5849.

[22] Yamauchi, K., Uenikawa, S., Enomotot, A., Tanimoto, H. *et al.*, Transglutaminase for reducing allergenicity of food proteins and/or peptides and methods for reducing their allergenicity, *Jpn. Kokai Tokkhyo Koho JP* 1991, 0327253.

[23] Watanabe, M., Suzuki, T., Ikezawa, Z., Arai, S., Controlled enzymatic treatment of wheat proteins for production of hypoallergenic flour, *Biosci. Biotechnol. Biochem.* 1994, *58*, 388–390.

[24] Adachi, T., Izumi, H., Yamada, T., Tanaka, K. *et al.*, Gene structure and expression of the rice seed allergenic proteins belonging to the alpha-amylase/trypsin inhibitor family, *J. Mol. Biol.* 1993, *21*, 239–248.

[25] Tada, Y., Nakase, M., Adachi, T., Nakamura, R. *et al.*, Reduction of allergenic proteins in transgenic rice plants by antisense gene, *FEBS Lett.* 1996, *391*, 341–345.

[26] Ogawa, T., Samoto, M., Takahashi, K., Soybean allergens and hypoallergenic soybean products, *J. Nutr. Sci. Vitaminol.* 2000, *46*, 271 – 279.

[27] Suszkiw, J., Researchers develop first hypoallergenic soy beans, *Agric. Res. Mag.* 2002, *50*, 16 – 17.

[28] Kato, T., Katayama, E., Matsubara, S., Omi, Y. *et al*., Release of allergenic proteins from rice grains induced by high hydrostatic pressure, *J. Agric. Food Chem.* 2000, *48*, 3124 – 3129.

[29] Jankiewicz, A., Baltes, W., Bögl, K. W., Dehne, L. I. *et al*., Influence of food processing on the immunochemical stability of celery allergens, *J. Sci. Food Agric.* 1979, *75*, 359 – 370.

[30] Hildebrandt, S., Steinhart, H., Paschke, A., Comparison of different extraction solutions for the analysis of allergens in hen's egg, *Food Chem.* 2008, *108*, 1088 – 1093.

[31] Hildebrandt, S., Kratzin, H. D., Schaller, R., Fritsché, R. *et al*., In vitro determination of the allergenic potential of technologically altered hen's egg, *J. Agric. Food Chem.* 2008, *56*, 1727 – 1733.

[32] Dube, M., Zunker, K., Neidhart, S., Carle, R. *et al*., Effect of technological processing on the allergenicity of Mangoes (*Mangifera indica* L.), *J. Agric. Food Chem.* 2004, *52*, 3939 – 3945.

[33] Hoppe, S., Neidhart, S., Zunker, K., Hutasingh, P. *et al*., The influences of cultivar and thermal processing on the allergenic potency of lychees (*Litchi chinensis* SONN.), *Food Chem.* 2006, *96*, 209 – 219.

[34] Schubert, S., Steinhart, H., Paschke, A., The influence of different potato (*Solanum tuberosum)* strains and technological processing on allergenicity, *Food Agric. Immunol.* 2003, *15*, 41 – 53.

