

Baking Additives as New Allergens in Baker's Asthma

X. Baur, W. Sauer, W. Weiss

Pneumologische Abteilung, Medizinische Klinik I, Klinikum Grosshadern, Universität München, FRG

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As reported by several authors [1-4], inhalative uptake of enzymes is associated with a high risk of developing allergic respiratory diseases. Awareness of this risk is especially important for the baking industry, where extensive use of various powered enzymes, predominantly from the genus *Aspergillus*, is common. These baking additives are added to flour for technical reasons, i.e. to improve the quality of bread, pastry, fancy cakes etc. (table I); in addition, they hasten and economize the baking process.

During the last 3 years we have observed an increasing number of patients with a typical history of baker's asthma who did not demonstrate any sign of the expected sensitization to flour allergens. Since, in earlier research we had shown that fungal α -amylase plays an important role in baker's asthma [5], we extended our previous studies and included as test substances in the routine diagnostic procedure (skin tests; radioallergosorbent test, RAST) a group of flour additives used by the patients at their place of work. Here we

report the results obtained with enzyme products and soybean flour which proved to be potent inhalent allergens.

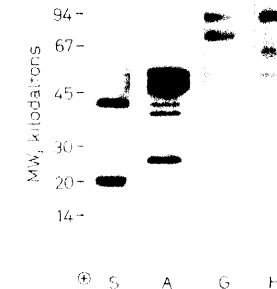
140 people who had been employed in the baking industry for at least 6 months and who suffered from workplace-related asthma, rhinitis and/or conjunctivitis were examined in this study.

Crude enzyme preparations (technical enzymes), such as those used at the place of work, were purified by high-performance column chromatography using a TSK-3000 column; fractions exhibiting enzymatic activity (estimated according to Bergmeyer [6]) were further investigated by RAST and immunoblot technique. The latter allows identification of IgE-binding components after their separation according to molecular weight using SDS-PAGE.

As shown in table I, the three *Aspergillus* enzymes which are currently in routine use in baking processes caused sensitization in 5-24% of this symptomatic group. Corresponding results were obtained with soybean flour which is also widely used.

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Fig. 1. Detection of immunologically reactive components of baking additives using immunoblot technique after SDS-PAGE. SDS-PAGE was performed on a 7-15% polyacrylamide gradient gel following the technique of Laemmli [7]. For blotting and immunological detection, samples were electrophoretically transferred onto nitrocellulose sheets with 25 mmol/l Tris, 192 mmol/l glycine containing 20% methanol and 0.1% SDS and incubated with patient's serum (0.5 ml) followed by ^{125} I-labelled anti-human IgE (250,000 cpm) and autoradiography (7 days) on X-ray film. MW = Molecular weight markers, stained with Amido black 10B; S-H = autoradiogram of a nitrocellulose sheet after incubation with a pool serum of 4 sensitized patients: S = soybean flour; A = α -amylase; G = glucoamylase; H = hemicellulase (partially purified; contains also cellulase activity).

**Table I.** Frequency of sensitization to baking additives

Enzyme	Frequency of sensitization, %	n	Use in baking industry
α -Amylase (<i>Aspergillus oryzae</i>)	24	33/140	provide fermentable sugars for yeast, improve loaf volume and texture
Glucoamylase (<i>Aspergillus niger</i>)	5	7/140	
Hemicellulase (<i>Aspergillus niger</i>)	8	11/140	
Papain (<i>Carica papaya</i>)	1	1/100	improve flavour and texture, preserve quality of biscuit crackers, control dough rheology
<i>Bacillus subtilis</i> protease	1	1/100	
Soy bean flour (contains lipoxigenase)	21	29/140	bleaches carotinoids in dough, improves dough rheology

serum demonstrates that each test substance contains at least two allergenically reactive components (fig. 1); bound IgE antibodies are seen as dark bounds.

Similar results were obtained with 20 individual sera of sensitized persons (not shown). However, there were some variations in the intensity and pattern of IgE reactivity within individuals. Inhalative challenge tests using these products resulted either in acute rhinitis ($n = 7$), immediate ($n = 8$) or dual asthmatic reactions ($n = 5$) in the 20 RAST-positive persons investigated, but no reactions were observed in the 5 RAST-negative controls.

Compared to the *Aspergillus* enzymes, sensitization to proteases from *Carica papaya* and *Bacillus subtilis* which are predominantly used in the production of biscuit and related foods, proved to be rare (table I).

Allergy to baking additives, and especially to enzymes from the genus *Aspergillus*, must be taken into account in the diagnosis of baker's asthma. Up to 24% of the symptomatic workers are sensitized to these enzymes and to soybean flour. Inhalation of such products must be minimized, particularly by using modernized processing methods, including ventilation, exhaust systems and the wearing of filter masks, in order to minimize the risk of allergic reactions to these potent inhalent allergens.

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Dr. X. Baur
Klinikum Grosshadern
Postfach 70 12 60
D-8000 Munich 70 (FRG)