

Heating inactivates the enzymatic activity and partially inactivates the allergenic activity of Asp o 2

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Background Sensitization to various flours and flour additives in the baking industry has been known for some time. However, most studies refer to allergens in their native state.

Objective The aim of our study was to find out how high temperatures during the baking process influence enzymatic and allergenic activities of the common flour additive alpha-amylase (Asp o 2), a relevant allergen for bakers derived from *Aspergillus oryzae*.

Methods In order to assess the allergenicity of Asp o 2 during the baking process, four to 11 correspondingly sensitized bakers were investigated by Enzyme Allergo-Sorbent Test (EAST) with native Asp o 2 and Asp o 2 heated to 80, 90, 95, 99.8 or 200°C. Furthermore, the enzymatic activity was assayed in simulated baking processes at the above mentioned temperatures.

Results Elevated temperatures resulted in a gradual loss of IgE antibodies' recognition of Asp o 2 in two commercially available enzyme products. The enzymatic activity decreased more rapidly. Heating the enzyme to 200°C abolished both the enzymatic and the allergenic activity of the enzyme.

Conclusion Based on these results, alpha-amylase in flour additives partially retains its allergenicity. This finding could be relevant for consumers.

Keywords: allergens, alpha-amylase, baker's asthma, occupational disease, heat inactivation, enzyme activity

Clinical and Experimental Allergy, Vol. 26, pp. 232–234. Submitted 17 May 1995; revised 19 July 1995; accepted 25 August 1995.

Introduction

Two case reports provide evidence of residual allergenicity of the widely used baking enzyme $\hat{A}$ -Amylase Asp o 2 [1] in bread and baking products [2,3]. Both subjects developed immediate-type respiratory symptoms and cutaneous hypersensitivity reactions. The aim of our study was to evaluate the inactivation of the IgE-binding capacity in relation to the enzymatic activity of Asp o 2.

Materials and methods

Enzymatic activity measurements of $\hat{A}$ -amylase products

The enzymatic activity of $\hat{A}$ -amylase preparations was

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estimated by the Phadebas, amylase test (Pharmacia, Uppsala, Sweden) according to the instructions of the manufacturer. Briefly, a water-insoluble crosslinked starch polymer carrying a blue dye was hydrolysed for 5 min at 37°C by $\hat{A}$ -amylase preparations or standards with defined activity forming water-soluble blue fragments. Absorbance was measured at 620 nm, and enzyme activity was indicated by the standard curve.

Enzyme products

We investigated commercially available $\hat{A}$ -amylases from *Aspergillus oryzae* with different degrees of purity with or without heat inactivation: $\hat{A}$ -amylase from *Aspergillus oryzae*, Novo Copenhagen, Denmark (N; enzyme activity 5758 PAU/g, Phadebas, Amylase Units; 196 mg protein/g, determined by the Bradford

assay [4] using bovine serum albumine as standard). $\hat{A}$ -amylase from *Aspergillus oryzae*, Miles-Kali, Hannover, Germany (K; enzyme activity 218 PAU/g; 20 mg protein/g solid). $\hat{A}$ -amylases from *Aspergillus oryzae* (Novo and Miles-Kali, heated in a water bath to 80°, 90°, 95° and 99.8°C for 10 or 20 min); $\hat{A}$ -amylase from *Aspergillus oryzae* (Miles-Kali) baked at 200°C over 50 min in an oven.

Skin-prick test

The skin-prick test reaction corresponding to the histamine weal was categorized as 3+ positive. A test result is regarded as positive if the weal diameter is at least half the size of that of the histamine reaction (2+).

Determination of specific IgE antibodies in serum by Enzyme Allergo Sorbent Test (EAST)

We dissolved the enzymes in 0.1 N bicarbonate buffer, pH 8.5, and coupled them after filtration to cyanogen-bromide-activated cellulose disks at a concentration of 1 mg/mL. Afterwards, specific IgE antibodies were determined in the usual way by EAST (Pharmacia, Uppsala). In accordance with common assessment procedures, we categorized values between 0.35 and 0.70 kU/L (class 1) as borderline findings and values above 0.70 kU/L as positive. At least 98% of 138 controls [5] were shown to always produce EAST values below this cut-off. The variation coefficient of interassay measurements was μ m 15%. EAST autoinhibition assays confirmed the specificity of IgE binding with inhibitions of >90%.

Patients

Sera from 11 bakers suffering from workplace-related

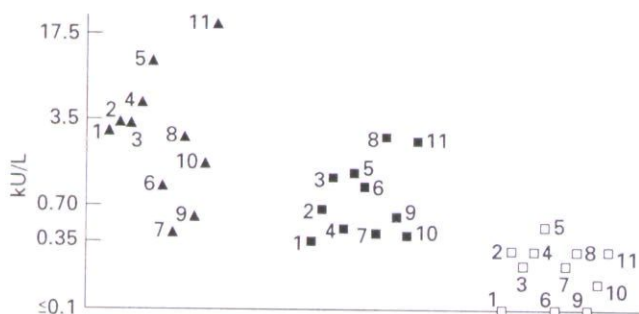


Fig. 1 IgE antibodies to native $\hat{A}$ -amylase and to $\hat{A}$ -amylase heated to 200°C for 50 min. Sera of 11 bakers sensitized to $\hat{A}$ -amylase as shown by skin-prick test were tested for specific IgE-binding to (▲) $\hat{A}$ -amylase of company N, to (■) $\hat{A}$ -amylase of company K and to (□) heated (200°C, 50 min) $\hat{A}$ -amylase of company K by EAST.

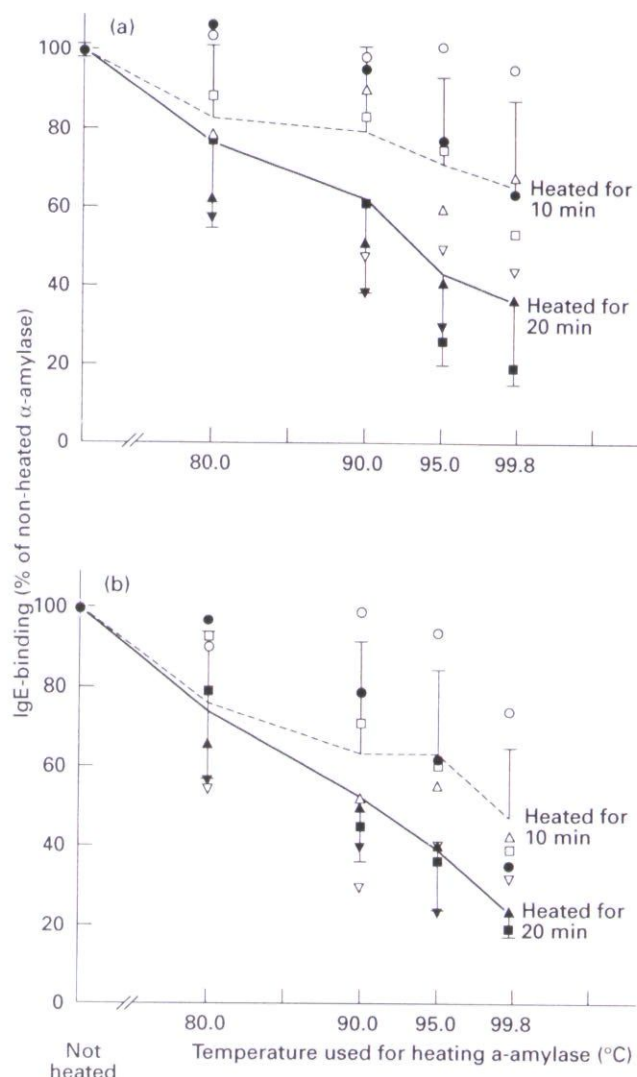


Fig. 2 Antibody binding capacity of native (non-heated) and heated $\hat{A}$ -amylase. $\hat{A}$ -amylase was heated for 10 min or 20 min to 80°C, 90°C, 95°C and 99.8°C and coupled to solid phase. With sera of four sensitized bakers, specific IgE-binding to non-heated and heated $\hat{A}$ -amylase was tested. Binding to heated amylase was shown in percentage of the binding to non-heated protein. Values of individual bakers are shown by the same symbol (○ for 10 min, ● for 20 min heated amylase). (a) The preparation of $\hat{A}$ -amylase from company N was used. Individual IgE values obtained with non-heated $\hat{A}$ -amylase (regarded as 100%) were 19.33 kU/L, 5.94 kU/L, 8.80 kU/L and 4.29 kU/L. (b) The preparation of $\hat{A}$ -amylase from company K was used. IgE values obtained with non-heated $\hat{A}$ -amylase (regarded as 100%) were 16.96 kU/L, 3.73 kU/L, 4.61 kU/L and 3.02 kU/L.

rhinitis and asthma showing at least a 2+ reaction in the skin test to $\hat{A}$ -amylase N (concentration 1 mg/mL) were used.

Results

Nine out of the 11 bakers showed a positive IgE-reaction to $\hat{A}$ -amylase N and five to $\hat{A}$ -amylase K (Fig. 1). All other subjects obtained borderline (class 1) EAST results. With the exception of one borderline case, no IgE binding could be detected after heating $\hat{A}$ -amylase to 200°C for 50 min.

Heated $\hat{A}$ -amylase additionally investigated in detail with the sera from four sensitized bakers showed a decrease in antibody binding capacity dependent on applied temperature and length of heating procedure (Fig. 2). After heating $\hat{A}$ -amylase for 20 min to 99.8°C, 19–63% of the initial IgE antibody binding were observed. Individual differences may be due to divergent antibody specificities involving various epitopes.

The enzyme activity was already reduced to levels below 15% at a temperature of 80°C and decreased to borderline or negative values at higher temperatures (Fig. 3).

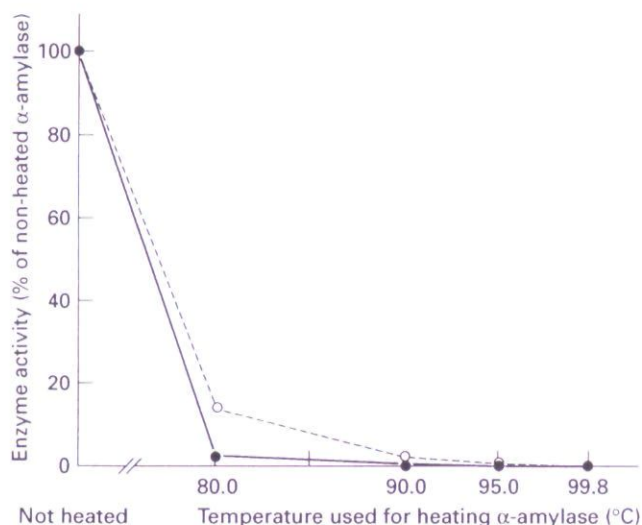


Fig. 3 Temperature-dependent inactivation of $\hat{A}$ -amylase from company N. $\hat{A}$ -amylase from company N was heated for 10 min (○) or 20 min (●) to 80°C, 90°C, 95°C and 99.8°C. The measured enzyme activity is shown in percentage of the value of the non-heated $\hat{A}$ -amylase. Corresponding tests with amylase of company K did not reveal a significant enzyme activity of any of the heated samples (always < 3% of the unheated product).

Discussion

Recent studies with the highly purified $\hat{A}$ -amylase (Asp o 2; MW 53 kDa) proved the active enzyme to be the allergenic component in commercial products prepared from *Aspergillus oryzae* cultures [1].

This study demonstrates that $\hat{A}$ -amylase heating to 80 and 99.8°C leads to a continuous decrease in IgE binding. After heating to 200°C, the binding capacity was almost nil. In comparison with this result, the enzyme activity is already widely eliminated at 80°C.

It should be mentioned that temperatures of dough and bread vary during the baking process. The temperature directly on the surface corresponds to the ambient temperature (in general 200–250°C) while the interior shows a mainly constant temperature of about 98°C owing to water vaporization [6,7].

Our latest investigations of clinically defined groups not occupationally exposed (consumers) have hitherto shown that the peroral uptake of partly denatured enzymes could result in clinically relevant hypersensitivity reactions. Due to the frequently difficult verification by anamnesis of the clinical relevance of food sensitization, supplementary oral provocation tests with different bread types baked with and without the enzymes should be performed in doubtful situations.

Acknowledgements

We thank Dr Pagel, Solvay Enzymes Hannover, for measuring enzymatic activities of $\hat{A}$ -amylase products.

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