MULTIPLE SUCCESSIVE IMMUNOPRINTING ON CYANOGEN BROMIDE-ACTIVATED NITROCELLULOSE MEMBRANES TO DETECT ANTIGENS AND ALLERGENS IN MEAT PRODUCTS

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1. INTRODUCTION

Allergy is a disease increasing in frequency, affecting 10 to 15% of the human population. Indeed, it is a major concern for the consumers. The detection of the allergenic constituents in modern food products can be uneasy due to their low concentration or to treatments involved in their preparation. Food ingredients or additives, often elaborated from plant, milk or egg protein origin, can be a potential risk for atopic subjects. Soluble extracts of raw, pasteurized or sterilized meat pastes containing hen's egg white were focused at their isoelectric points in agarose gel and then immobilized by multiple successive transfers onto cyanogen bromide-activated nitrocellulose membranes. Antigens and allergens of egg white were detected either by rabbit anti-egg white antiserum or by sera from egg white sensitive patients.

2. MATERIALS AND METHODS

Rabbit anti-hen's egg white antiserum was produced by CTSCCV. Human sera were obtained from egg white sensitive patients whose RAST (RadioAllergoSorbent Test, Cap System, Pharmacia, Uppsala, Sweden) were of class IV. Meat pastes containing the equivalent of 2% egg white dry powder used as a binding agent were stored raw (R) or were pasteurized (P) or sterilized (S).

Isoelectric focusing (IEF) on agarose gel was performed. The separated molecules were then immobilized by multiple successive transfers onto cyanogen bromide-activated nitrocellulose membranes. After incubation with secondary antibodies conjugated to alkaline phosphatase (Sigma Chemical Co, St Louis, MO, USA), antigens and allergens were visualized using NBT/BCIP substrate.

3. RESULTS

The egg white antigens detected by the rabbit antiserum are mainly lysozyme ovotransferrin and ovalbumin (figure 1). Egg white antigens are recognised in the crude binding agent (E3) Ingredient and In the raw or pasteurised meat products, but not in the sterilised meat product.

The allergen patterns recognised by two sensitive patients are shown on figure 2 and 3. Ovalbumin and ovomucoid are allergenic molecules for the H [1] x E patient serum. H [4] x E serum recognises only ovotransferrin as an allergen.

4. CONCLUSION

The multiple successive immunoprint technique carried out allowed to visualise egg white antigens and allergens in ingredients and in raw or pasteurised products, even at low concentrations. An exact superposition of the antigen and allergen patterns is possible by this technique. ELISA methods should be used for sterilised products when immunoprint techniques are not sensitive enough.

Ingredients used in agro-food industry may contain allergenic molecules. Prevention of food allergic disorders needs improved methods of analysis and a complete information of the consumers on the product labels.

5. REFERENCES

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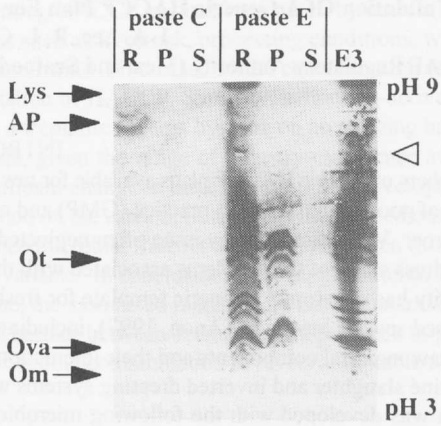

Figure 1. Egg white antigens revealed by rabbit anti-egg white antiserum. Meat paste C: negative control which does not contain any binding agent; meat paste E contains 12% pasteurized - freezed egg white (E3); R: raw; P: pasteurized; S: sterilized. AP: endogenous meat alkaline phosphatases; Lys: lysozyme; Ot: ovotransferrin; Ova: ovalbumin; Om: ovomucoid. ◁ : sample application.

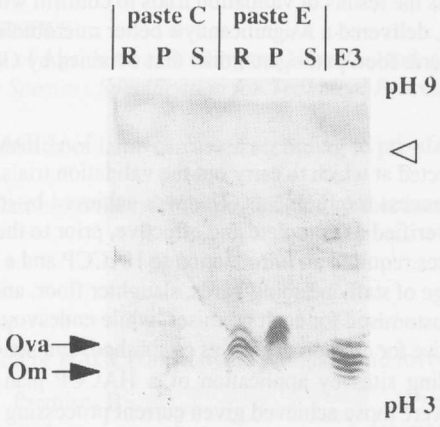

Figure 2. Egg white allergens revealed by the IgE of H [1] x E human serum. Meat paste C: negative control which does not contain any binding agent; meat paste E contains 12% pasteurized - freezed egg white (E3); R: raw; P: pasteurized; S: sterilized. Ova: ovalbumin; Om: ovomucoid. ◁ : sample application.

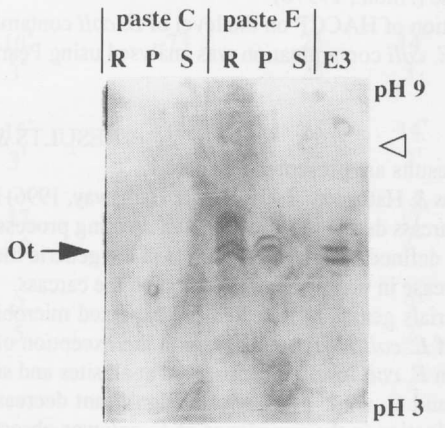

Figure 3. Egg white allergens revealed by the IgE of H [4] x E human serum. Meat paste C: negative control which does not contain any binding agent; meat paste E contains 12% pasteurized - freezed egg white (E3); R: raw; P: pasteurized; S: sterilized. Ot: ovotransferrin. ◁ : sample application.