

Decrease of the Allergenic Activity of Foods by Shock Waves

N. WOLFF^{1*}, U. COGAN¹, H. ZUCKERMAN¹, N. KARIN², Y. LEVY³, Y. E. KRASIK⁴,
J. FELSTEINER⁴, R. REIFEN⁵ and S. YANNAI¹

¹Faculty of Biotechnology and Food Engineering, ²Faculty of Medicine and ⁴Faculty of Physics,
Technion – Israel Institute of Technology, Haifa, Israel, *E-mail: wnnafi@bezeqint.net;

³Kipper Institute of Immunology, Schneider Children's Medical Center, Petah-Tikva, Israel;

⁵Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University
of Jerusalem, Rehovot, Israel

Abstract: Food allergy significantly affects the life-quality for many people worldwide and is life-threatening in extreme cases. In recent years the incidence of this disease shows a gradual increase in many countries. It is a well-established fact that common food-processing operations involving heat treatments fail to significantly decrease the allergenic reactivity of foods. Furthermore, allergenic proteins are remarkably resistant to proteolysis by digestive enzymes and often remain intact after passing through the gastrointestinal tract. The tested materials were protein extracts from sesame seeds, milk and peanuts, or isolated proteins from the same sources. Treatments investigated included application of 6 to 10 pulses of high-frequency acoustic shock waves, lasting a few microseconds. The treated samples were tested *in vitro* by Western blotting with sera from humans diagnosed to be allergic to the food in question, and *in vivo* by measuring the IgE levels produced in young Brown Norway rats exposed to the tested proteins by sensitisation through i.p. injection, or by feeding for up to 6 weeks, using direct and indirect ELISA. The treatments markedly decreased or completely eliminated the allergenic reactivity of the foods, as evidenced by the assays used.

Keywords: food allergy; shock waves; sensitisation; IgE levels

INTRODUCTION

There is a growing concern about food allergy, due to its increased incidence [1] and the severity of its clinical manifestations. Hence, the food industry must meet the needs of allergic consumers. A variety of methods have been tried, attempting to decrease the allergenicity of foods, especially thermal treatments, but few techniques have been reported to be successful. Some allergenic foods are heat-stable, while others are partially-stable or labile. Thermal processing can create new epitopes, exposed by unfolding or by reactions with other food compounds, as well as destroy existing epitopes [2, 3]. Therefore, the question whether and how heat treatments may alter the allergenicity of food is very complicated [4].

The aim of our study was to reduce the allergenicity of protein extracts from sesame seeds, milk and peanuts, or isolated proteins from the same sources, by shock wave treatment. To-date, shock wave treatments were only used for control of microorganisms [5, 6] and for protein modification [7].

EXPERIMENTAL

Subjects. Forty seven subjects were included, all of whom showed immediate allergic reactions, following the ingestion of milk, peanuts or sesame-containing foods within 2 h after ingestion. Clinical manifestations observed included cutaneous and respiratory symptoms, as well as anaphylaxis. They were subjected to a skin prick test (SPT), in order

to verify previous sensitisation to the tested foods [8]. Ten healthy subjects, with no history of allergic reactions, were selected as negative controls. Sera from all subjects were collected and stored at -70°C until used. Detection of IgE specific to protein extracts from sesame seeds, milk and/or peanuts was performed with the Pharmacia CAP System.

IgE binding assay. Western blot tests [9–10] with serum samples, of the different sesame, milk and peanut proteins, before and after treatments, were performed for assessment of their allergenicity. Quantification of the intensity of IgE binding of a specific serum sample to a specific protein was carried out by densitometry.

Animals. Brown Norway rats were reared on a commercial diet devoid of sesame, milk and peanuts. Pre-study blood samples were always tested for sesame, milk and peanuts-specific antibodies.

Experimental design. 4–5 weeks old animals, were fed untreated or treated sesame seed protein extract for 6 weeks (1 mg protein per rat/day). Negative control animals received the sesame-free rodent diet, and positive control animals received untreated sesame seed protein extract. The experimental groups received sesame protein extract treated by

shock waves (6 pulses). After a 6-week feeding, the animals were returned to the negative control diet for 2.5 months, during which their sesame-specific IgE decreased to background levels. We then performed cross-over feeding between the groups: positive control animals received sesame seed protein extract treated by shock waves. The experimental groups received untreated sesame protein extract for 6 more weeks (1 mg protein per rat/day). Blood samples were collected from the animals at prespecified time intervals, and sera were separated and kept frozen for ELISA testing of specific IgE levels.

Shock wave treatments. The experimental set-up consisted of two $0.5\ \mu\text{F}$, 50 kV Maxwell low-inductance capacitors, charged in parallel by a high-voltage power supply. This system generates pulsed discharge ($\sim 3\ \mu\text{s}$ each) creating a high pressure ($\sim 1100\ \text{atm}$). A single treatment included 6–10 pulses for sesame, and 10 pulses for the milk and peanut samples.

RESULTS AND DISCUSSION

Sixteen of the 17 sesame-allergic subjects showed a positive reaction with the untreated major allergen β -globulin, and 6 showed a positive reaction

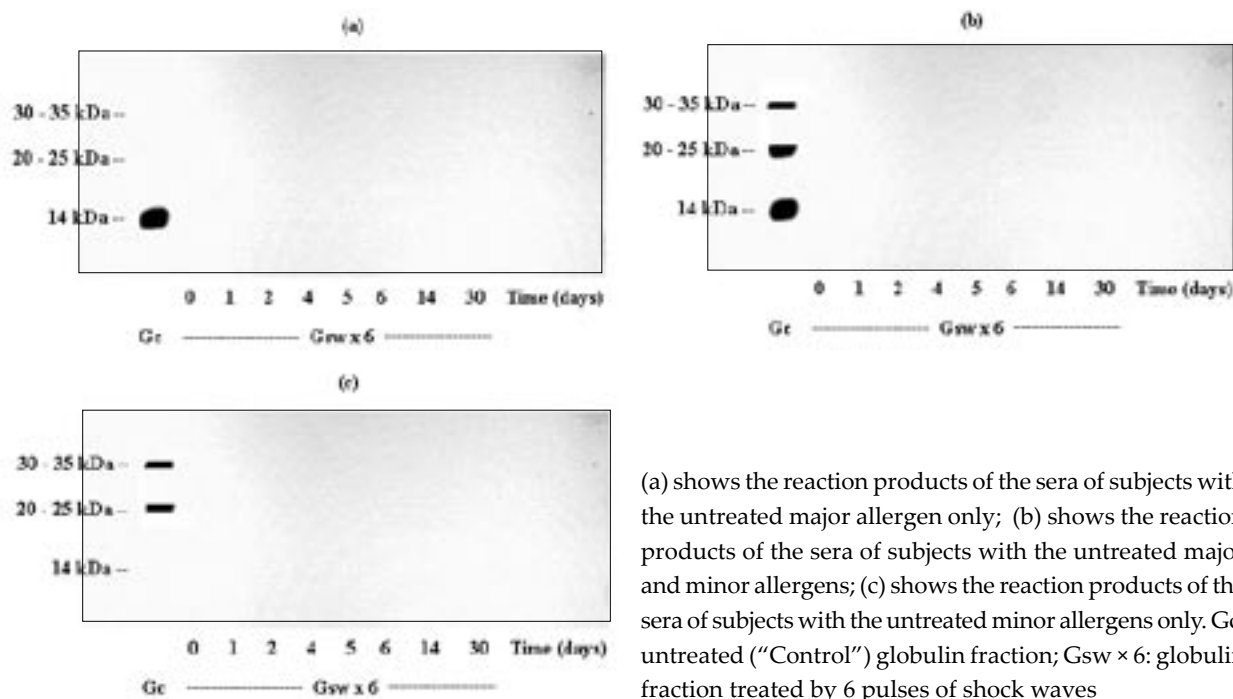


Figure 1. Testing for regeneration of allergenicity of the treated sesame proteins by shock waves, 30 days following treatment

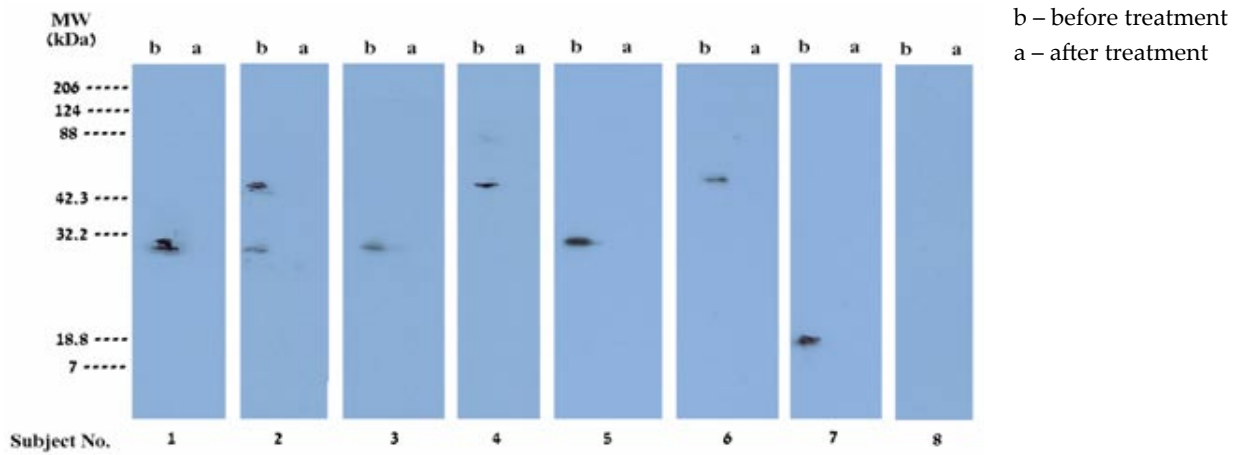


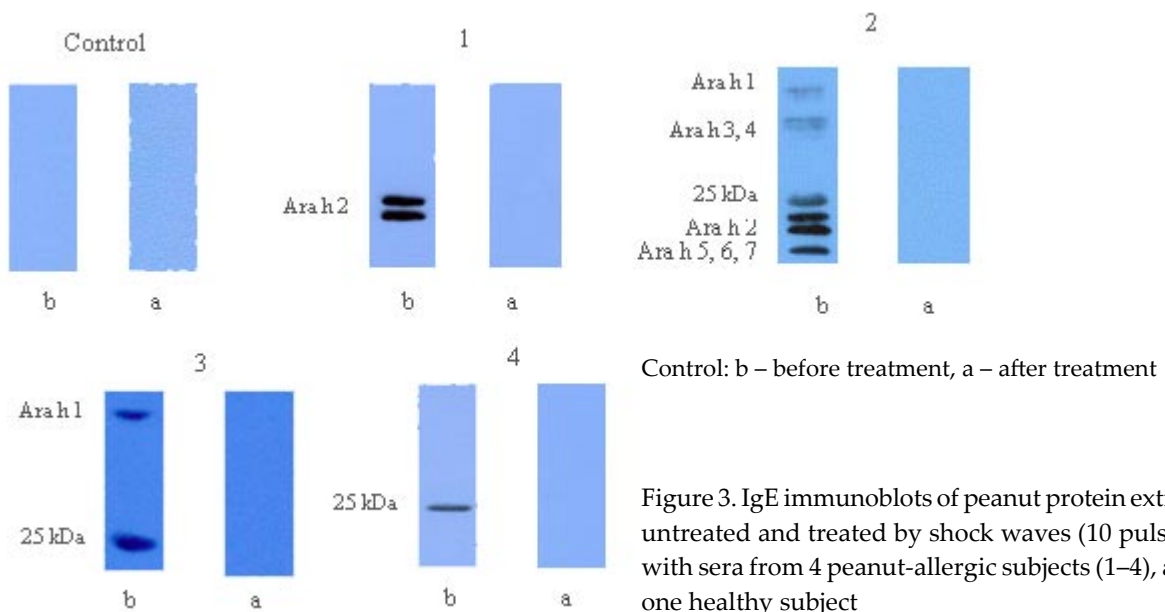
Figure 2. IgE immunoblots of untreated and treated (by 10 pulses of shock waves) reconstituted skim milk powder with sera from 7 milk-allergic subject and one healthy subject

with the untreated minor allergen α -globulin. The application of shock waves resulted in elimination of all allergenic potential, except in one serum sample, which indicated the presence of a small residual allergenicity. Furthermore, no regeneration of allergenic activity of the sesame proteins treated by shock waves was detected up to 30 days following the treatment (Figure 1). Sera of the 20 milk-allergic individuals exhibited IgE binding to different proteins of the untreated reconstituted milk powder, 7 cases of which are presented in Figure 2. IgE binding to the shock-wave treated proteins was largely abolished as shown for the same 7 cases (Figure 2). Similar results were observed with other 8 sera samples.

With 5 sera samples only partial (3 cases), or no effect (2 cases) were observed.

Complete and partial abolishment of the allergenic reactivity was observed also in the case of IgE binding to shock-wave treated extract of peanut proteins (Figure 3).

Figure 4 shows the rats' response to exposure to the various untreated and treated sesame proteins in terms of their specific IgE levels. It appears from the results that: The shock wave treatment resulted in a complete elimination of specific IgE in the sera of most rats fed the treated sesame protein extract. The inactivated allergenicity of the sesame proteins failed to undergo regeneration within 14 days following the shock wave treatment. It is



Control: b – before treatment, a – after treatment

Figure 3. IgE immunoblots of peanut protein extract untreated and treated by shock waves (10 pulses), with sera from 4 peanut-allergic subjects (1–4), and one healthy subject

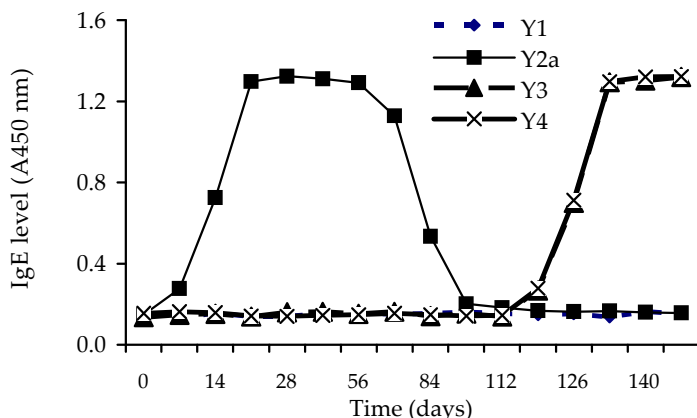


Figure 4. Follow-up on the IgE level in representative BN rats from each group, that were exposed to the sesame globulin fraction before and after shock waves treatment. The IgE level in BN rats, that were exposed in the first or the second stage to the globulin fraction treated by shock wave.

Y1 – Negative control group; Y2a – Positive control group; Y3 – Group receiving the treated protein extract; Y4 – Group fed the treated protein extract 14 days after the shock-wave treatment

likely that the reduction/elimination of the allergenic reactivity due to shock-wave treatment, is associated with conformational changes in these food proteins.

CONCLUSIONS

1. The shock wave treatment resulted to a large extent in reduction/elimination of the allergenic activity of the 3 foods investigated.

2. The effectiveness of the treatment was equally demonstrable in both human and Brown Norway rats, proving the validity of our *in vivo* rat model for the prediction of potential allergenicity of foods for humans.

Acknowledgements: The work with sesame was funded by the Israeli Ministry of Health, and the work with milk and peanut products by the European Commission.

References

- [1] ORTOLANI C., PASTORELLO E.A. (1997): Eur. Commission Sci. Res.: 58.
- [2] DAVIS P.J., WILLIAMS S.C. (1998): Allergy, **53**: 102.
- [3] BERRENS L. (1996): Allergy Net.: 277.
- [4] HANSEN K.S., BALLMER-WEBER B.K., LUTTKOPF D., SKOV P.S., WUTHRICH B., BINSLEV-JENSEN C. (2003): Allergy, **58**: 132.
- [5] LOSKE A.M., PRIETO F.E., ZAVALA M.L., SANTANA A.D., ARMENTA E. (1999): Shock Waves, **9**: 49.
- [6] ZUCKERMAN H., KRASIK YA.E., FELSTEINER J. (2002): Innov. Food Sci. Emerg. Technol., **3**: 329.
- [7] KIMELDORF M., GLEIZER S., KRASIK YA.E., FELSTEINER J., BRUMFELD V., ZUCKERMAN H. (2003): Innov. Food Sci. Emerg. Technol., **4**: 151.
- [8] DALAL I., BINSON I., REIFEN R., AMITAI Z., SHOHAT T., RAHMANI S., LEVINE A., BALLIN A., SOMEKH E. (2002): Allergy, **57**: 362.
- [9] TOWBIN H., STAEHLIN T., GORDON J. (1979): Proc. National Acad. Sciences U.S.A., **76**: 4350.
- [10] BURNETTE W.N. (1981): Anal. Biochem, **112**: 195.