

# Allergologia et immunopathologia

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ORIGINAL ARTICLE

# Vinegar decreases allergenic response in lentil and egg food allergy

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#### **KEYWORDS**

Food allergy; Gastric digestion; Acid-suppression medication; Vinegar

#### **Abstract**

*Background*: Food allergy results from an atypical response of the mucosal immune system to orally consumed allergens. Antacid medication inhibits the digestion of dietary proteins and causes food allergy. A decrease of the gastric pH might enhance the function of digestion and reduce the risk of food allergy.

*Objective*: To test a possible decrease in the allergenicity of powerful food allergens (egg, chicken, lentils) with the addition of vinegar during the cooking process.

Methods: We included seven patients who suffered from anaphylaxis due to egg, chicken and lentils. We added vinegar to egg, chicken and lentil processed extracts used for skin prick tests (SPT) and compared the wheal areas obtained with the same extracts sources and the same way but without vinegar addition. Immunodetection was performed with the different processed extracts and patients' sera. Only one patient consented food challenge with vinegar-marinated-chicken.

Results: Wheal areas were significantly minor with the food extract with vinegar. Inmunodetection showed a decrease of the response with vinegar processed extracts. Conclusions: Vinegar addition during the cooking process may decrease lentil and chicken allergenicity.

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#### Introduction

Gastric digestion substantially decreases the potential of food proteins to bind IgE, which increases the threshold dose

of allergens required to elicit symptoms in patients with food allergy<sup>1</sup>. The ability of food proteins to remain structurally intact after gastrointestinal digestion increases their potential to be allergens<sup>1</sup>. Impairment of gastrointestinal digestion with antacid medication represents a risk factor for food allergy induction<sup>1,2</sup>. By increasing the gastric pH, they interfere substantially with the digestive function of the stomach, leading to persistence of labile food

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proteins during gastric transit. On the contrary, a decrease in gastric pH perhaps might improve food digestibility and decrease their allergenicity.

Skin prick testing is a rapid screening method for IgE-mediated food hypersensitivity. Positive skin tests may be helpful especially when a clear-cut history of food reactivity is present<sup>3</sup>.

Most studies have used raw foods as starting material for skin prick testing, thus not considering potential changes in allergenic properties induced by the cooking process (dressing, marination) and other industrial processing to produce derived foodtuffs. Recent studies showed that the processing of wheat flour to obtain derived foodstuffs seems to decrease strongly the IgE binding capacity of the major salt-soluble wheat proteins. Moreover, simulated gastric fluid digestion could further inactivate some heat-resistant potential allergens<sup>4</sup>.

Vinegar is a wine-derived product widely used to improve food digestibility but its potential capacity to change the allergenicity of food has not been measured.

A young patient, who suffered from severe sensitisation to egg and chicken (anaphylaxis), maintained that she tolerated chicken marinated previously in vinegar (a usual practise in the Mediterranean area, where it is commonly added to legumes dishes). This prompted us to perform a study in patients who had suffered from anaphylaxis due to ingestion of powerful food antigens such as egg, chicken and lentils, using extracts of previously cooked food like those usually consumed by our patients.

#### Patients and methods

We included seven patients who had suffered from anaphylaxis due to egg, chicken and lentils. These patients were selected from the database of food allergic patients (Hospital Rio Hortega, Valladolid, Spain). They had all been diagnosed by prick tests (SPT), specific IgE, and double-blind

placebo-controlled food oral challenge to the food implicated

Specific IgE antibodies to egg and egg allergens, chicken, lentil and other legumes and a battery of foods were determined using the Immuno-CAP 100 System (Phadia AB, Uppsala, Sweden). Demographic and clinical data of selected patients can be seen in Table 1.

Informed consent was obtained from each patient and ethical approval from the Ethical Committee of the Hospital Rio Hortega.

### Skin prick tests

Skin prick tests were performed with a commercial panel of food allergenic extracts (Bial-Aristegui Laboratories, Bilbao, Spain). In the cases of egg allergy we also tested egg allergens, (ovoalbumin, ovomucoid, connalbumin, chicken albumin) and in lentil also the lentil pets (*Bruchus lentis*). Additionally we boiled 100 g of chicken meat during 30 min in water and performed two extracts 1/10 w/v from the same preparation (3.4 mg of protein/ml). In one of the extracts we added 5 ml of vinegar from white wine. The pH of these extracts (measured with Acilit<sup>®</sup>, Merck, German) was 4.2 in the case of lentil and 4.5 in the case of egg. These extracts were tested in controls (10 atopic and 10 nonatopic) in order to exclude irritative responses to vinegar.

In the same way we boiled 200 g of lentils with and without vinegar. The two extracts had 2.8 mg of protein/ml.

SPT were done with all the extracts and wheals measured after 15 min and outlined by tracing on adhesive paper. Saline solution 0.9% was used as negative and histamine  $(10\,\text{mg/ml})$  as positive control. A wheal area greater than  $7\,\text{mm}^2$  was considered positive.

#### Oral challenge tests

Only one patient (sensitised to chicken and egg) consented to double-blind placebo-controlled food challenge with

Patient n.	Age/sex	Reported symptoms	Specific IgE			SPT					
			Lentil (kU/l)	Other fo (slgE > 0.		Lentil (mm²)	Lentil + vinegar (mm²)	Soy (mm²)	Green pea (mm²)	Peanut (mm²)	
8742	3/M	Ad, U	3.08	egg, tuna		28.26	8.2	28	78.5	32	
9750	2/M	Ad, An	7.35	egg, cod,		78	26.6	19	78.5	68	
8949	3/F	As, An	22.3			62	15.6	65	42	48	
8816	3/F	As, An	1.61	ba, ry, co		28,5	12.8	32	28	28	
8805	25/M	An, U	0.82	ba, ry,		38	10.6	28	22	22	
6306	46/M	An	27.01	ba, ry		69	19.9	60	62	62	
10168	30/F	An	17.2	ba, ry, so	OV	62	12	43	12	36	
			Egg white	E. yolk, chick		Egg white	Egg white+ vinegar	Chicken	Chicken+ vinegar		
10165	21/F	An	>100	>100	92	142	41	99	15		
10162	9/M	An	17.8	15	19.21	42	12	30	10		

As, asthma; An, anaphylaxis; U, urticaria, Atopic dermatitis; ba, barley; ry, rye; ri, rice; co, corn.

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vinegar-marinated-chicken. The possibility of oral challenge in all patients was difficult because of the severity of their symptoms.

#### In vitro tests

IgE-Immunoblots were performed with and without vinegar added to the allergenic sources (lentils and chicken) under non-reduced conditions with a pool of sera from the patients, diluted 1:10.

#### Results

Measurements of SPT areas and IgE levels can be seen in Table 1 and Figure 1.

Double-blind placebo controlled challenge was negative with 10 g of chicken with vinegar and positive (urticaria-angioedema) after eating 2 g of chicken meat without vinegar.

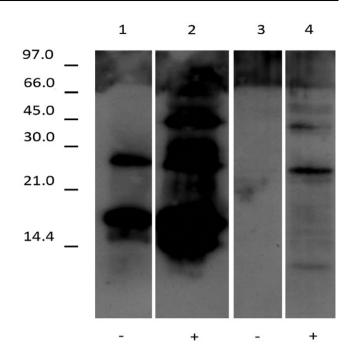
Immunoblots revealed less specific IgE binding to the lentils and chicken with vinegar than to the lentils and chicken without vinegar. The vinegar added to lentil proteins reduced the IgE-binding to the proteins between 30 and 90 kDa with respect to lentils without vinegar. No bands were recognised by the pool patients' sera to chicken with vinegar with respect to chicken without vinegar Figure 2).

## Discussion

Digestion assays with simulated gastric fluid have been performed in order to characterise food allergens. So, true food allergens (digestion-resistant class 1) trigger direct oral sensitisation, and labile class 2 allergens, that are heat labile and susceptible to digestive processes, are nonsensitising elicitors. Infants have augmented vulnerability because of decreased acid production and reduced pancreatic and intestinal enzymatic activity<sup>5</sup>. Consequently, they have an increased absorption of intact food proteins, which may cause stimulation of the immune system and generation of IgE antibodies.



**Figure 1** Skin prick tests with lentil extracts with and without vinegar.



**Figure 2** IgE-Immunoblotting. Pool of sensitised patient's sera against: lane 1: lentils with vinegar, lane 2: lentils without vinegar, lane 3: chicken with vinegar, lane 4: chicken without vinegar.

Alterations in the gastric milieu are frequently experienced in the very young and in the elderly or as a result of gastrointestinal pathologies. Additionally, acid-suppression medications are frequently used for treatment of dyspeptic disorders. By increasing the gastric pH, they interfere substantially with the digestive function of the stomach, leading to persistence of labile food protein during gastric transit. Indeed, both murine and human studies reveal that antacid medications increase the risk of food allergy induction<sup>1,2</sup>. Gastric digestion substantially decreases the potential of food proteins to bind IgE, which increases the threshold dose of allergens required to elicit symptoms in patients with food allergy.

Vinegar added in processed food makes the pH more acid and may help the gastric labour. In popular medicine its digestive effects have been well-known for a long time. It is also known that at low pH levels, the acidic amino acid residues in the active digestive pepsin moieties undergo protonation. The electrostatic interactions between the N-terminal prosegment and the active pepsin are disrupted, which initiates a conformational change in both the prosegment and the active enzyme portion. Thus, the removal of the prosegment results in conversion into the enzymatically active form of pepsin<sup>6</sup>. Whereas at a pH above 5.0, limited pepsin is activated, the rate of active enzyme increases with decreasing gastric pH. An acidic milieu is required for the proteolitic activity of pepsins, with an activity optimum between pH 1.8 and 3.2. Here we have demonstrated a significant decrease both in skin response and 'in vitro' response to the same allergens after vinegar addition. Taking into account not only the rising number of food-allergic patients but also the severity of food-induced adverse reactions, a simple and reliable method like the addition of this popular food dressing may be useful in certain cases of food hypersensitivity.

#### Conflict of interest

The authors have no conflict of interest to declare.

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