Allergy to beer and wine caused by *Saccharomyces cerevisiae* in a patient sensitized to fungi

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Beer and wine are the most widely consumed alcoholic beverages in the world. Wine is made from fermented grape juice and beer is brewed from cereal grains (most commonly malted barley) fermentation. Hops are also used to flavor the beer. Both are produced by fermentation with yeasts (*Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*). Hypersensitivity reactions to beer or wine are rare and have been mainly attributed to grains [1] or grapes. Proteins from barley are the most common cause of beer allergy [2].

The presence of potential barley allergens and proteins from *S. cerevisiae* have been described in beer by proteomic studies (nsLTP, gliadins, glutelins, trypsin alfa-amylase inhibitors, serpins) [3, 4] and some of them have been pointed out as the triggering agents of the allergic reaction in some cases: nsLTP (9 kDa), protein Z (45 kDa) [5] and protein z-type serpin (20-25 kDa) [6]. In the same way, yeast sensitization [7] has been described as the cause of allergy to beer, cider, and wine.

A 33-year-old woman with a personal history of allergic rhinoconjunctivitis and exercice induced asthma. Since three years ago she had suffered several episodes of anaphylaxis (ocular pruritus, eyelid angioedema, pharyngeal occupation, dysphonia, diarrhea, urticaria and dizziness) with no associated exercise. These reactions were resolved with self-administered epinephrine. She related the episodes after the ingestion of beer with chips or olives. Occasionally she had suffered milder symptoms after drinking red and white wine. She tolerates other alcoholic beverages, cereals including bread, grapes, nuts and all kind of food.

Protein extracts from different brands of beer (Heineken®, San Miguel®, Chimay®, Franziskaner®) were prepared by homogenization in phosphate-buffered saline (50% V/V) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5), dialyzation against destilled

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water and liophilization. As some beers are produced with wheat, one of the studied beer was a wheat beer (Franziskaner®).

Skin prick tests (SPT) with common aeroallergens (pollens, dust mites, molds, pet dander) were positive to *Dermatophagoides pteronyssinus* (3x3), *Lepidoglyphus destructor* (3x3), *Alternaria alternata* (3x3) and *Aspergillus fumigatus* (7x8). SPT were positive with beer extracts: Heineken® (5x5), San Miguel® (4x4), Chimay® (6x5) and Franziskaner® (5x5) (from wheat); red wine (7x7), white wine (8x6), *Saccharomyces cerevisiae* (7x6), raw *Saccharomyces cerevisiae* (6x6), cooked *Saccharomyces cerevisiae* (6x6), *Penicillium nalgiovense* (7x6) and mushrooms (8x8). SPT performed with cereal extracts (wheat, barley, corn), fruits (apple, peer, peach, red and white grape) and Pru p 3 (peach nsLTP) were negative.

Total serum IgE (ImmunoCAP Thermofisher) was 178 kU/l and the results of specific IgE were: 4.51 to *Saccharomyces cerevisiae*, 4.29 to *Penicillium chrysogenum* (*P. notatum*), 3.93 to *Aspergillus fumigatus*, 3.87 to *Candida albicans*, 1.93 to *Cladosporium herbarum*, 1.93 to *Alternaria alternata*, 4.24 to rPru p 3 and 3.96 to rMal d 3. Specific IgE <0.10 to cereals (barley, oat, maize, malt, hop, rye, wheat, rTri a 19, rTri a 14), rAlt a 1, rAsp f 2, rAsp f 4 and rAsp f 6.

SDS-PAGE immunoblotting was carried out under reducing conditions (with mercaptoethanol) as described by Lammeli [8], with Franziskaner® and Chimay® beer extracts, *Saccharomyces cerevisiae* extract and patient serum (Figure 1). In order to study the possible involvement of the cereal nsLTP in the allergic reaction due to beer ingestion, the beer extracts were also incubated with a rabbit serum against Pru p 3.

A similar profile of IgE reactive bands were detected in both beer extracts, being the main ones bands around 97 kDa, 80 kDa, 55 kDa, 40 kDa, 32 kDa and 17 kDa. In *S. cerevisiae* extract a high intensity IgE-binding zone was revealed between 100 – 29 kDa and also a band of around 17 kDa was detected. The anti-Pru p 3 rabbit serum revealed a band with molecular mass below 14 kDa in both beer extracts, no bands of this molecular mass was detected in beer extracts with the patient serum.

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In order to study if *S. cerevisiae* was the allergenic source of the IgE reactive proteins detected in beer extracts, an immunoblotting-inhibition assay was carried out with Chimay® beer extract in solid phase and beer extracts and *S. cerevisiae* extract as inhibitors. Both beer extracts as well as *S. cerevisiae* extract produced a total IgE-binding inhibition on Chimay® beer extract.

A case of allergy to *Saccharomyces cerevisiae* in a patient who suffered from anaphylactic reactions by beer, red wine and sauces has been published [9]. The authors suggested that it may be due to cross-reactivity with antigens from fungi to which the patient was sensitized (*Cladosporium herbarum, Alternaria alternata, Aspergillus fumigatus, P. notatum, Malassezia furfur* and mushroom).

Proteins 5 to 100 kDa have been described in beer, mainly albumins, globulins, serpin, amylase inhibitors, lipid-binding proteins, chaperones and enzymes. During the manufacturing process, proteins can also be modified. The proteins most frequently found in beer are Serpin-Z-4 (45 kDa) and LTP (9 kDa) [10]. The result obtained by SDS-PAGE immunoblotting suggests that those proteins are not involved in this case, as they are not recognized by the patient´s serum. Proteins of 97 kDa, 80 kDa, 55 kDa, 40 kDa, 32 kDa and 17 kDa from *Saccharomyces cerevisiae* not described before, might be the responsible for the patient's condition.

We present a case of beer and wine allergy caused by *Saccharomyces cerevisiae* allergy which is used in the fermentation of both beverages. When this allergenic source undergoes a heating process it does not cause allergy symptoms. We believe that some *Saccharomyces cerevisiae* allergens can be inactivated with heat as our patient tolerates bread ingestion. She had a previous history of respiratory allergy due to *Alternaria alternata* and subsequently she developed anaphylactic reactions after drinking beer and wine. Therefore, we believe that the primary sensitization could be due to environmental fungi. More research will be necessary in the future to identify and characterize allergenic proteins of *Saccharomyces cerevisiae.*

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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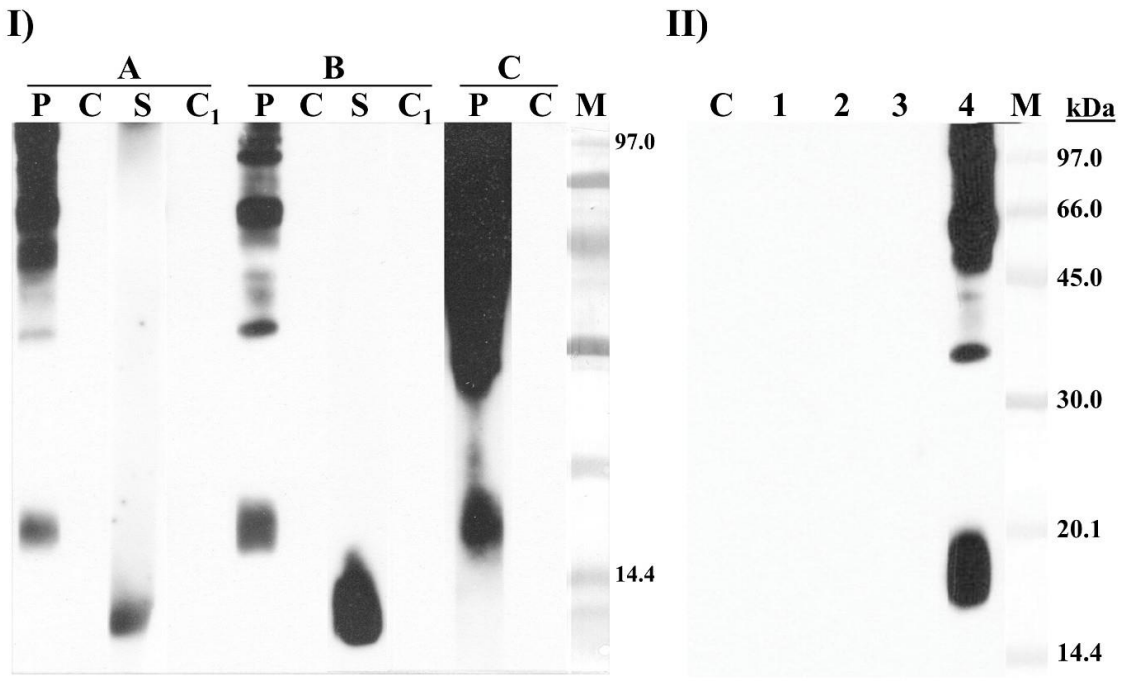
Figure 1. I) SDS-PAGE Immunoblotting. A) Franziskaner® beer extract B) Chimay® beer extract C) *S. cerevisiae* extract. Lane P: Patient serum, Lane C: control serum (pool of sera from non atopic subjects), Lane S: Anti-Pru p 3 rabbit serum, Lane C_1: Unimmunized rabbit serum, Lane M: Molecular mass standard. II) SDS-PAGE Immunoblotting – inhibition. Solid phase: Chimay® beer extract. Lane C: control serum (pool of sera from non atopic subjects), Lane 1 – 4: patient serum preincubated with Chimay® beer extract (lane 1), with Franziskaner® beer extract (lane 2), with *S. cerevisiae* extract (lane 3), with lamb extract (lane 4).



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