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In Europe, the application of lupine seeds to produce food has increased significantly in recent years. Lupine (flour, seeds or dust) can induce different allergic responses. Consumption of lupine-fortified products may also provoke allergy syndromes. This article reviews the adverse reactions to lupine, after various contacts with this plant, including eating lupine products and seeds. It discusses cases which confirm properties of lupine protein as a primary allergen. It describes lupine protein cross-reactivity and the modifying effect of thermal processes on lupine protein.

INTRODUCTION

There is no doubt that a diet has a significant impact on our health. For years we could observe changes in traditional proportions of consumed food with increasing consumption of food articles of plant origin. Legumes are usually recognized as the richest source of vegetable proteins. The high nutritional value of this plant is attributed to: amino acid protein composition similar to the composition of animal proteins and a high nutritional value of lipid fraction. Legumes are rich in many valuable compounds of which: oligosaccharides, phenol compounds, tocopherols, fibre and phytoestrogens deserve to be mentioned here [Cantoral *et al.*, 1995; Mazur *et al.*, 1998; Lampart-Szczapa *et al.*, 1997, 2003a,b; Makri *et al.*, 2005; Duranti, 2006].

One of the representatives of the *Leguminosae* family, lupine has been used as a food component for many years in many countries of South America (in Chile and Peru lupine is being consumed as an easy and cheap source of protein) and recently also in Australia. On the other hand in France, the Netherlands, Italy, Spain and Germany, lupine-fortified products have appeared on the market quite recently and are becoming more and more popular because of their high nutritional value and functionality. Lupine is added mainly to baker's products and pastry, and is also applied in dietary and functional foods [Faeste *et al.*, 2004]. However it must be mentioned here that in many countries, where lupine has already been accepted by consumers there are reports about allergic reactions following consumption of lupine-containing products.

LUPINE AS A PRIMARY SENSITIZING FACTOR

Growing incidence of legume allergy is observed because of its increasing consumption [Holden *et al.*, 2005; Peeters

et al., 2005]. In countries where lupine has already become an accepted ingredient of food products investigations of its sensitizing properties pose a challenging scientific problem.

Lupine – inhaled and contact allergen

Below we are going to present a few documented examples which confirm that lupine proteins can be contact allergens.

Gutierrez and his co-workers [Gutierrez *et al.*, 1997] reported in 1997 a case of urticaria in a 25-year old man after kissing his girlfriend who had earlier eaten lupine seeds. The patient was found to suffer from: erythema, severe itching and progressive onset of wheals round the contact area. In clinical tests the same symptoms occurred but only in the case of direct and indirect contact with chewed, moist seeds.

There is evidence supporting allergenic potentials of lupine resulting from particles of lupine infiltrating into human organism, through the respiratory system. The first case of respiratory symptoms after inhalation of lupine particles was described by Novembre *et al.* [1999]. It occurred in 3-year old child with a history of episodic asthma. When the child was playing with the dust of lemon tree (manured with ground lupine), he reacted with a severe asthma attack including: rhinorrea, conjunctivitis, cough, cyanosis and dyspnea. Skin prick tests were performed. Most of them were negative with regard to a number of inhaled and food allergens. Skin prick test, *in vitro* IgE binding test (CAP RAST system) and provocation test responses to lupine extracts, were strongly positive. After exposure to lupine in powder form the child responded with: rhinitis, conjunctivitis, cough and wheezing lasting 3 minutes. Presumably the protein fraction of about 45 kDa is the principle antigen recognized by the patient's antibodies.

In 2005, a case was reported of an asthma attack in an 8-year old child with peanut hypersensitivity [Moreno-

Ancillo *et al.*, 2005]. He was taken to clinic with the suspicion of an adverse drug reaction due to salbutamol. The boy suffered the asthma attack while playing with his 14-year old brother who was eating lupine seeds as a snack. This 14-year old boy recognized the symptoms of asthma and administered - without washing hands, a portion of salbutamol, to his sick brother. Unexpectedly the asthma attack worsened. The skin tests were positive with *Lupinus albus* extract, peanut, garbanzo bean, navy bean, green bean, pea, lentil and soy bean. Positive responses were shown in serum specific IgE tests by *Lupinus albus*, peanut, soy, lentil and garbanzo bean. In challenge tests with inhaled salbutamol, the patient tolerated well this drug, but he had asthma attack 5 minutes after he was exposed to lupine seeds. It was concluded that it was lupine dust hovering in the air during their games that was the cause of the initial asthma attack.

Occupational lupine allergy

Parisot *et al.* [2001] were among the first researchers who reported occupational lupine allergy. They examined a 30-year old woman (an environmental technician who complained of: rhinitis, conjunctivitis and palpebral angioedema). She failed to use gloves while preparing lupine flour for tests. Earlier the patient had never reacted allergic. Prick test, serum specific IgE binding protein test, were positive with flour from lupine seeds. The woman stopped working with lupine flour which also ended her problems with lupine allergy.

In the same year, Crespo and co-workers [2001] evaluated the influence of lupine on employees working with lupine. They performed investigations including 7 women (about 35 years of age) who had worked with lupine seed flour at a laboratory for 15 years. Three of the patients had respiratory problems which began less than an hour after they had contact with lupine flour. During the last 18 months, one of them developed occasional ocular itching when handling lupine flour. The second one, more or less during the same period of time, experienced multiple episodes of nasal rhinorrhea, sneezing, lacrimation and ocular itching which usually occurred already after 5 minutes of work with lupine and kept for two hours. The third worker, with almost 20-year experience, reported: chest tightness, wheezing, cough, rhinoconjunctivitis, 10 minutes, after she or someone near her, started to work with lupine. They never sought medical consultation because of work-related symptoms. The results of immunologic tests and inhalation challenges confirmed an occupational allergy to lupine seed flour in 2 of the 7 subjects. Allergic sensitization to lupine probably developed *via* the respiratory tract. The same patients show allergic symptoms also after eating lupine proteins.

It is clear from the above remarks, that lupine seeds – particularly in flour form, should be treated as a factor which can trigger off an allergic reaction by inhalation and which additionally can result in the development of occupational asthma and food allergy.

Allergy syndromes after eating lupine and its products

At present, Australia is the biggest lupine producer and that is probably why problems of lupine-related allergies are most acute there. Smith *et al.* [2004] reported three cases of allergy

after eating lupine-fortified products. Urticaria, angioedema, cough, respiratory difficulty, developed right after eating products containing lupine bran. The reactions were so severe that they required hospitalization. Two of the three patients, had earlier seasonal pollen allergy and thus had an increased propensity to develop food allergy, while the third one had no previous allergies at all. None of them was allergic to peanut. The consumption of the meal which turned out to be harmful was not accompanied by activities which could have increased mucosa permeability of the alimentary tract and enhanced the probability of allergic reaction.

THE RISK OF CROSS-REACTIVITY AMONG PROTEINS OF LEGUME FAMILY

Eating foods which contain legumes involve danger of allergic cross-reactions. When studying the molecular basis of cross-reactions, it was found that amino acid sequences of storage proteins of this botanical family were similar [Shutov *et al.*, 2003]. In addition, high homology and frequent presence of proteins acting as protease inhibitors was also observed [Mello *et al.*, 2001; Paiva *et al.*, 2006]. It turned out that lectins present in many legumes can also participate in the reaction with IgE antibodies [Shibasaki *et al.*, 1992; Burks *et al.*, 1994; Larsson, 2006], so it is quite probable that also lupine proteins can contribute to allergic reactions by cross-reactivity.

Hefle *et al.* [1994] were among the first who reported lupine cross-reactivity with peanuts. Urticaria and angioedema occurred in the 5-year old girl with peanut sensitivity after eating a spaghetti-like pasta fortified with sweet lupine seed flour. The pasta extract was analysed with the skin test and *in vitro* serum specific IgE test. The experiment included six adult patients (aged 27-48) with peanut sensitivity, to check whether eating pasta involved allergic reactions. In five of the seven subjects, skin test results were positive to the lupine pasta extract. Those individuals reported a history of green pea sensitivity. Immunoblotting studies showed that the serum IgE from peanut sensitive patients bound to a band at 21 kDa and somewhat weaker to several bands with molecular weight ranging from 35 to 55 kDa.

In 1999, in France, a group of scientists studied lupine allergenicity in patients allergic to peanut [Moneret-Vautrin *et al.*, 1999]. They were the first to apply, except skin prick tests, also the labial challenge test and the double-blind placebo-controlled food challenge (DBPCFC). The skin prick test results with lupine were positive in 11 of 24 patients (44%). Six children underwent the DBPCFC, two others – the labial challenge test; seven of them had clinical symptoms.

The same year, Leduc *et al.* [1999] characterised cross-reacting lupine proteins. Cross-reactivity with peanuts concerned proteins with molecular weight in the range of 43-45 kDa and 65 kDa. The 43-45 kDa fraction was found the strongest allergenic.

An event was reported in Norway, where a 24-year old woman allergic to peanut, experienced in four separate situations, an allergic reaction after eating a certain brand of hot dog bread (swelling of the lips, urticaria and rhinoconjunctivitis) [Faeste *et al.*, 2004]. The patient was examined in order

to identify the allergen that evoked such undesirable response. The extract from the hot dog bread, peanut and lupine were immunochemically analysed with the patient serum and polyclonal anti-lupine antibodies (derived from blood of immunized rabbit). The results indicated that this type of bread contains lupine proteins or perhaps contains proteins with immunoreactivity similar to lupine proteins (it was surprising because lupine-containing products are not popular in Norway). The tests failed to confirm the presence of peanut antigens in this kind of bread and the authors concluded that cross-allergenicity of peanut and lupine was of clinical significance. After the bread producer was informed about the incident, he confirmed the use of lupine flour to baking and included appropriate information on the product's label.

An article was published in a French medical journal reporting a case of acute asthma in a young girl allergic to peanut [Kanny *et al.*, 2000]. In the past she repeatedly experienced severe asthma attacks with symptoms of anaphylaxis caused by peanuts. She was proposed a restriction diet deprived of peanuts. The applied diet had a positive effect and the girl had no asthma attacks for over 7 months. Afterwards she underwent control tests during which her reaction to raw and cooked lupine extract was analysed. Skin prick and serum specific IgE tests were positive. The oral challenge test was also performed which resulted in larynx itching, lips painfulness and dyspnea one hour and half after eating the last portion of lupine. The girl was administered appropriate medicine, the symptoms receded only to come back one and quarter hours later. This time the reaction included: shortness of breath, speaking inability and low respiratory efficiency. The reactionary dose of lupine was small (965 mg) so it was recognized that the consumption of lupine flour in baker's products brings high risk for patients with peanut allergy.

In August 2004, a 25-year old woman experienced anaphylaxis after eating a meal of chicken, French-fried potatoes and onion rings [Radcliffe *et al.*, 2005]. During the meal her tongue and lips started to swell and 15 minutes later, her swelling throat impeded breathing and caused overall weakness. The patient had a history of asthma and anaphylaxis after eating peanut, so it was expected that the severe reaction was elicited by peanut contamination in the meal. The restaurant chief excluded such a possibility and suggested contact with onion rings distributor. There they confirmed the use of lupine flour during the manufacturing process. Skin prick and serum specific IgE tests to lupine were carried out and the results were strongly positive. The patient refused to undergo the oral challenge.

Also Matheu and co-workers [1999] reported an anaphylactic episode after eating lupine seeds in a patient with peanut tolerance. Right after a 38-year old atopic woman ate three lupine seeds, she suffered urticaria, dysphagia, chest tightness, shortness of breath and throat swelling as well as angioedema on her hands and face. In her past she reacted allergically also to chick pea, lentil and white bean. During skin prick tests with lupine ocular itching and palmar erythema appeared. During 5 years, that is from the moment the patient first reported food allergy symptoms, clinical cross-reactivity developed progressively. At first, her symptoms appeared only after eating chick pea, but later, she reacted allergically also to lentil, white bean, lupine and pea.

Guarneri and co-workers [2005], found a high sequence homology between pathogenesis-related protein PR-10 of white lupine and allergen Ara h 8 of peanut. Also another lupine protein, β -conglutin precursor, shows significant homology with the Ara h 1 allergen of peanut. Authors used computer-aided amino acid sequence comparison and three-dimensional modeling. They suggest that PR-10 and β -conglutin of white lupine cross-react with Ara h 8 and Ara h 1 of peanut, respectively. These two lupine proteins could be responsible for allergic reactions.

In 2005, Magni *et al.* [2005] identified IgE-binding polypeptides of *Lupinus albus*. They assessed also IgE cross-reactivities with other legume species. One- and two-dimensional gel electrophoresis and immunoblotting analyses showed that two lupine proteins: conglutin γ and 11S globulin basic subunits strongly react with sera of all lupine sensitive patients. It was observed cross-reactivity with other legume seed protein extracts, containing such polypeptides. Authors concluded that these mentioned polypeptides from *L. albus* may represent allergens.

IMPACT OF THERMAL PROCESSING ON LUPINE ALLERGENICITY

The immunogenicity of the raw material is also affected by the type of the applied technological processes. This is connected with the applied processing conditions which can exude one of the three effects: leave the obtained product unchanged, or increase, or decrease its allergenicity [Davis *et al.*, 2001; Taylor & Lehrer, 1996].

The allergic reaction is usually caused by a small fragment (epitope) of the food protein polypeptide chain. The minimum number of amino acid residues in a linear epitope is 8, while a three-dimensional conformational epitope consists of at least 16 amino acid residues [Taylor & Lehrer, 1996].

According to Besler *et al.* [2001] changes in protein conformation that occur during food processing, may either destroy the existing epitopes on a protein surface or may generate new ones – so-called “neoallergens”.

Most food products are subjected to thermal processing during their production and at home. Thermal processing (essential and profitable) may, in many ways, modify food allergenicity. It is often thought that thermal processes decrease allergenicity, because high temperatures normally cause a disruption of protein structure. Unfortunately high temperatures may induce many complex physical and chemical reactions and initiate neoantigens [Davis *et al.*, 2001].

Little is known about the impact of thermal treatment on the allergenicity of lupine proteins. Álvarez-Álvarez *et al.* [2005] studied the effect of boiling, autoclaving, extrusion cooking on the allergic potential of lupine. They performed a series of analyses: immunoblotting with specific IgE, CAP Inhibition Assays and skin tests (serum samples were obtained from 23 patients with lupine allergy). The results suggest that lupine allergens are heat stable. An important reduction of immunoreactivity was observed only when lupine extracts were autoclaved at 138°C for 20 minutes. The authors believe that the application of the last treatment may decrease the allergenicity of lupine.

Allergens hidden in food are particularly dangerous for people with food allergy and that is why it is so important to include appropriate information on product labels. In Europe a system of mandatory labeling of twelve important allergens (irrespective of their quantity) have been in force since autumn 2005. The list is systematically re-examined and updated on the basis of the most recent scientific knowledge. On 23 December 2006 the European Food Safety Authority (EFSA) added lupine to the list in Annex IIIa of Directive 2000/13/EC [Directive 2006/142/EC]. Main reason of this decision was the relatively high risk of cross-allergy to lupine in between 30% and 60% of persons who are allergic to peanuts.

SUMMARY

The range of lupine-containing products continues to increase because of the functionality and nutritional value of this ingredient. Consumers choose legume foods more often than before. Cases of intolerances caused by eating lupine are still rare. However, we should be sensitive to this problem, because it might become important with increased use of lupine in many food products.

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