

Unsuspected Lupin Allergens Hidden in Food

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Key Words

Hidden allergens • Lupin • Food allergy

Abstract

Background: Lupin is a herbaceous plant from the legume family whose seed allergens usually have cross-reaction with peanut. Lupin flour is used in human nutrition because of its high nutritional and functional qualities. **Aims:** The aim of this work was to detect non-specified lupin proteins contained in several manufactured foods. **Methods:** Serum from a patient suffering anaphylactic episodes after ingestion of a certain brand of cookies and with oral allergy syndrome after eating chicken bouillon was used as a tracer. Lupin seeds and commercial food extracts were analyzed by SDS-PAGE, immunoblotting and immunoblotting inhibition. Lupin extract allergenicity after thermal processing was also analyzed. **Results:** A lupin allergen with a molecular weight close to 14 kDa was detected in extracts from cookies, a chicken bouillon cube and a chicken dehydrated soup. **Conclusions:** The presence of unsuspected, hidden non-specified lupin sources in food labeling was demonstrated. According to the results of this study, it is important for food-allergic patients that food labels should declare all the components irrespective of their quantity.

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Introduction

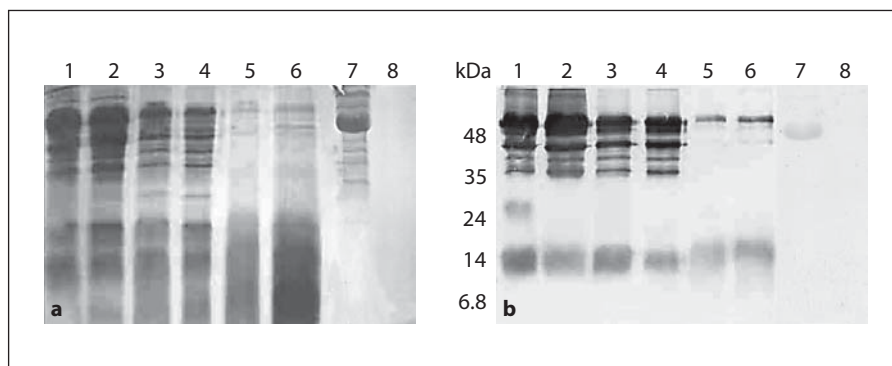
Lupinus albus is a herbaceous plant from the legume family used mainly in animal feed. Its ground seeds mixed with cereal flours are also used to make bread, cookies, pizza, pasta and milk substitutes. Lupin is used as an emulsifier in food manufacturing because of its high protein content. It has a high content of lysine and is low in sulfated amino acids, being an excellent wheat flour complement. Additionally, it has a high fiber content, is low in oil, and it is gluten free [1].

Lupin usually appears as an unlabeled ingredient in manufactured food. This is because manufacturers only label the contents of ingredients if a single component constitutes more than 5% of the total weight of the food. Besides, lupin is not considered one of the so called 'allergenic products' for which there is statutory labeling in the new legislation [2].

Some IgE-binding proteins of lupin seeds with molecular weights between 14 and 75 kDa have been described. Presumably, a protein with 43 kDa is responsible for the cross-reaction with peanut [3]. In addition, some of the lupin allergens seem to be heat resistant [4], a fact that increases their clinical relevance.

The aim of this work was to demonstrate the presence of hidden lupin allergens in several foods.

Fig. 1. SDS-PAGE (**a**) and immunoblot (**b**) of a lupin extract after thermal processing. Lane 1 = Crude lupin extract; lane 2 = crude lupin extract in reducing conditions; lane 3 = lupin extract after boiling; lane 4 = lupin extract after boiling in reducing conditions; lane 5 = lupin extract after roasting; lane 6 = lupin extract after roasting in reducing conditions; lane 7 = crude soybean extract used as control; lane 8 = PBS used as control.



Material and Methods

Antigen

Crude extracts from lupin seeds and commercial foods were performed as follows.

Lupin Extract. After soaking in water for 2 days, the seed coats were removed manually. Thereafter, the seeds were ground in a mortar. Protein was extracted (1:10 w/v) by shaking in phosphate-buffered saline (PBS) for 2 h. After centrifugation at 4,500 g for 15 min, the supernatant was collected and centrifuged again at 16,000 g for 15 min.

Commercial Food Extracts. Different soups, chicken bouillon cubes and cookies were ground to a smooth paste. These extracts were defatted by two washings with 10 volumes of cold acetone and dried overnight. Protein was extracted from dried powder as described above.

PBS and soybean extracts were used as controls.

Serum

Serum from a subject monosensitized to lupin with a positive 11-mm prick test, not sensitized to peanut, who had suffered two anaphylactic episodes when eating certain brands of fried tomato sauce and cookies, and had an oral allergy syndrome caused by ingestion of a brand of chicken bouillon cube was used for immunoblotting assays.

SDS-PAGE and Immunoblotting

Proteins from extracts were separated on a 16% acrylamide SDS-PAGE performed at 150 V under standard conditions [5]. After running, nitrocellulose sheets were placed on both sides of the gel and transfer was performed by diffusion for 18 h. After washings and blocking for 30 min in 3% NP-40 in PBS, the serum was incubated for 18 h while shaking. Thereafter, the membranes were washed and then incubated with 10 ml of a 1/1,000 dilution of a mouse monoclonal human anti-IgE antiserum for 3 h (Ingenasa, Madrid, Spain). After new washings, the membranes were again incubated with 10 ml of a 1/2,500 dilution of an alkaline-phosphatase-labeled goat anti-mouse IgG (Biosource Int., Camarillo, Calif., USA) antiserum for 1 h. Finally, the membranes were washed and the substrate (BCIP-NBT) was added and incubated for 30 min [5].

Antigen Treatments

Lupin seeds were treated as follows.

For heat treatment, lupin seeds were incubated for 20 min in a boiling water bath or roasted in a domestic oven at 170°C during 20 min as described by Beyer et al. [6].

For reduction, 20 µl of the crude, boiled and roasted supernatant extracts was mixed with the same volume of sample buffer with 1% 2-mercaptoethanol.

Immunoblotting Inhibition

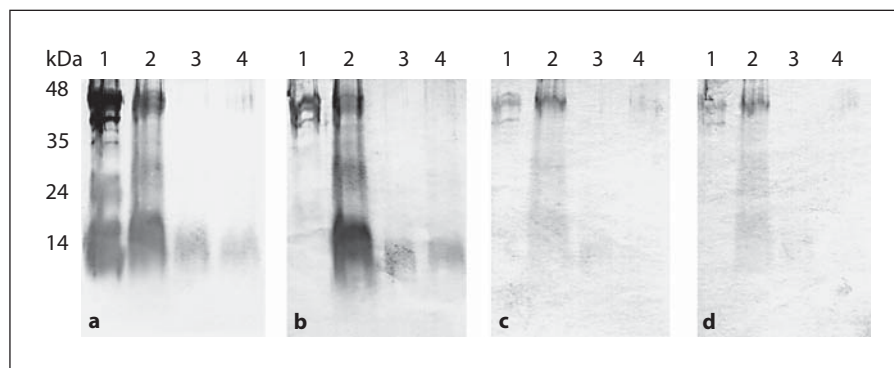
Immunoblotting inhibition was done to verify whether the 14-kDa protein detected in cookies, chicken bouillon cube and chicken dehydrated soup extracts was the same in all cases. The different lupin, cookies, bouillon cubes and soup extracts were separated by SDS-PAGE and transferred to nitrocellulose membrane as described above. Serum from the patient was incubated for 2 h with crude, boiled and roasted lupin extracts. Thereafter, the mixtures were incubated overnight with the nitrocellulose membrane, and the blot procedure was followed as indicated above.

Results

Specific IgE to proteins from the lupin seed extract were detected by the tracer serum. Allergens with a molecular weight between 48 and 14 kDa were identified (fig. 1b). After heat treatments and reduction with 2-mercaptoethanol, SDS-PAGE and immunoblotting were performed with crude, boiled and roasted protein extracts from lupin. As shown in figure 1b, heat treatment did not destroy the recognition of some IgE-binding proteins. A low molecular weight allergen of 14 kDa was highly resistant to heat and could therefore be potentially relevant for sensitized patients. Reducing conditions did not modify the IgE-binding protein recognition.

The two controls were negative for the recognition of the 14-kDa band.

Fig. 2. Lane 1 = Immunoblotting inhibition assay with extracts of crude lupin seeds; lane 2 = cookies; lane 3 = chicken bouillon cube; lane 4 = chicken dehydrated soup. **a** Incubation with the tracer serum. **b** Serum inhibited with a crude lupin extract. **c** Serum incubated with a boiled lupin extract. **d** Serum incubated with a roasted lupin extract.



When immunoblot was performed with commercial foods, IgE-binding proteins of similar molecular weight as those present in lupin were detected. Figure 2a shows that all the commercial extracts tested contained an IgE-binding protein of around 14 kDa, and this allergen was the only protein detected in two of the foods tested.

When the serum was incubated with crude lupin seed extract (fig. 2b), almost all the proteins of lupin disappeared, and only faint bands of 48 kDa remained. No inhibition was obtained in the lanes corresponding to food extracts. However, when the inhibition assay was performed by incubation of the tracer serum with a boiled or roasted lupin seed extract, the specific IgE detection to the 14-kDa allergen disappeared (fig. 2c, d).

Discussion

Lupin seed contains several types of storage proteins called conglutins [1]. Conglutins α include proteins with sedimentation coefficients of about 11–12S. They are referred to as 11S or ‘legumin-like’ globulins and consist of hexamers of two disulfide-linked heterogeneous subunits indicated as acidic (of a molecular weight ranging from 54 to 47 kDa) and basic subunits (20 kDa), respectively. Conglutins β have sedimentation coefficients of about 7S. They are classified as 7S or ‘vicilin-like’ globulins, and their trimeric structure consists of several polypeptides of 20–80 kDa, with no disulfide bridges. Conglutin γ is a basic tetrameric protein with sedimentation coefficient 7S, consisting of disulfide-linked 30- and 17-kDa polypeptides. Conglutin δ is a monomeric minor protein with a sedimentation coefficient of 2S made up of disulfide-linked polypeptide chains of 9 and 4 kDa [7]. The Ara h1 peanut allergen has strong sequence homologies with lupin conglutin β [1, 8]. The Ara h2 peanut al-

lergen has strong sequence homologies with lupin conglutin δ [1, 9, 10].

Anaphylaxis after eating lupin has been reported [11] and can be due to cross-reaction in people with peanut allergy or may arise de novo in people with no previous food allergy [12]. A major lupin flour allergen (molecular mass 43 kDa) is completely inhibited in immunoblotting by peanut extracts as described by Moneret-Vautrin et al. [3]. This 43-kDa polypeptide might coincide with the unreduced conglutin γ [10]. The 65-, 38- and 13-kDa lupin allergens were not inhibited [3]. Only one case of isolated allergy to lupin flour without pre-existence of peanut allergy has been reported [11].

In our study, a lupin allergen with a molecular weight of approximately 14 kDa was detected in several brands of manufactured food extracts that had not been described until now (cookie, chicken bouillon cube and chicken dehydrated soup). Magni et al. [10] described a lupin 2S protein of similar molecular weight. This 14-kDa protein modifies its allergenicity after heat treatment as shown in inhibition assays, but it does not change under reducing conditions.

In conclusion, we suggest that lupin should be considered in cases of unexplained food allergy or food allergy cases related to the above mentioned foods, and should be added to the list of ingredients requiring mandatory allergy warning labeling [2].

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