Investigation of Lupine Allergen Presence in Some Food Products by Enzyme-Linked Immunosorbent Assay

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RESEARCH ARTICLE

Abstract

Lupine is a legume and important hidden food allergen. In the present study, our aim was to investigate the presence of lupine in some foodstuffs including 90 packaged food samples (breakfast cereals, oatmeals, biscuits, and chocolate) belonging to different firms in Turkey. Extraction and determination of lupine in all samples were made by the enzyme-linked immunosorbent assay (ELISA) technique. The presence of lupine was detected in 11 (12.22%) of 90 food samples. Lupine was not detected in the samples of breakfast cereal and oatmeal. The mean levels (±S.E) of positive lupine of biscuits and chocolate samples were found to be 1.53±0.18 mg kg⁻¹ and 2.04±0.16 mg kg⁻¹, respectively. It was declared on the labels of some of the analyzed products that they may contain traces of lupine. Lupine was detected in only one of these samples. There was no information about the presence of lupine on the labels of other lupine detected samples. As a result, the importance of the detection of allergen substances in terms of food safety, food quality, and public health and the importance of food labeling are emphasized in this study. Analytical methods for monitoring allergens in risky foods are important.

Keywords: food allergen; lupine; ELISA

INTRODUCTION

Food consumption is one of the main activities in human life (Żukiewicz-Sobczak et al., 2013). One of the most common diseases associated with the consumption of some foods is a food allergy that is defined as an adverse health effect resulting from a specific immune response that occurs reproducibly when exposed to a particular food (Taylor, 1980; Hoyos-Bachiloglu et al., 2014). Food allergy is associated with low quality of life, limited social interactions, severe allergic reactions, potential death risk, and high socioeconomic status (Hoyos-Bachiloglu et al., 2014). It has been reported that an allergic component can cause a wide variety of symptoms and disorders and lead to life-threatening consequences (Sarinho and Lins, 2017). Management of food allergy is based on strict avoidance of allergenic foods (Devdas et al., 2018; Do et al., 2018; Sheridan et al., 2020). Accidental ingestion of allergens is common worldwide (Sheridan et al., 2020). Lupine is an important legume that can cause serious allergic reactions. Lupine is in the category of “risky allergens”. The prevalence of lupine allergy varies depending on dietary habits and geographical differences (Villa et al., 2020b). It belongs to the Leguminosae family (Lima-Cabello et al., 2019). The plant that is most produced worldwide and has the highest protein content among legumes is soybean (Aguilar-Acosta et al., 2020). However, lupine, which has been discovered in recent years, is preferred over soy in the food industry because it has 2-3 times more protein content (28-45%) than cereals and is well adapted in areas where soy and other legume seeds cannot be grown (Yorgancilar et al., 2020). More than 400 species of the genus Lupinus are known (Kohajdová et al., 2011). Lupinus albus, Lupinus luteus and
Lupinus angustifolius, known as sweet lupine, are regularly used in the food industry (Gayraud et al., 2009). Lupine is widely used as a food and ingredient in various processed products such as snacks, bakery products, meat, and dairy products due to its nutritional value and technological properties (Villa et al., 2020a). The prevalence of lupine allergy in the population is unknown. However, it is reported that the incidence of lupine allergy increases with the increase in lupine consumption. Lupine allergy can result in anaphylactic shock or death (Koeberl et al., 2018). Accurate identification of allergenic components in food products is very important in terms of avoiding allergic hazards. Commercial food products, such as packaged or processed foods, are a part of most consumers' diets because they not only have appropriate quality and shelf life but also provide convenience to consumers (Do et al., 2018). Undeclared allergens in packaged foods pose a serious health risk for sensitive individuals (Sheridan et al., 2020). There are legal regulations on food labeling in different countries. For the safety of food-allergic consumers, the transition of allergens to the product along the food supply chain, including cross-contact, should be monitored as well as proper allergen labeling practices (Do et al., 2018). Managing the risk posed by food allergens can be more difficult than by toxic chemicals or microbial pathogens. Food allergens cannot be an additional substance or pollutant, but they are also included in nutritious and common foods. For this reason, it is necessary to inform consumers and allergic patients by ensuring that food labels are complete, clear, and correct (Gendel, 2013). It is aimed to protect sensitive consumers with food labeling regulations. Enforcement of labeling legislation plays an important role in preventing the occurrence of adverse immunological reactions resulting from the accidental consumption of allergic foods (Villa et al., 2020b). Accurate, clear, and truthful product labels are very important for consumers with food allergies (Bedford et al., 2017).

The aim of the present study was to determine the presence of lupine in some packaged foodstuffs including breakfast cereals, oatmeals, biscuits, and chocolate obtained from Ankara local markets in Turkey. Also, this study aims at investigating the importance of the detection of lupine allergen substances in terms of food safety, food quality, and public health, and the importance of food labeling is emphasized in this study.

MATERIALS AND METHODS

Sample Collection
In this study, a total of 90 food samples including 31 breakfast cereals (A, B, C, D), 11 oatmeals (E, F), 33 biscuits (G, H, I, J, K) and 15 chocolates (L, M, N) from different firms were collected from local markets in Ankara, Turkey. Ninety food samples were produced in Turkey, except for G firm, and each has different serial numbers from different firms. However, these firms are also sold almost all over Turkey. Samples were collected between April 2021 and September 2021. These samples were collected before their expiration date. Lupine was declared on the labels of some food products that may contain traces of lupine. There was no information about the presence of lupine on the labels of other samples.

Analysis of Food Samples
Quantitative analysis of lupine proteins was determined with a sandwich ELISA using the Ridascreen® Fast Lupine/ELISA kit (R-Biopharm AG, Darmstadt, Germany). This assay was performed according to the guidelines of the manufacturers. Sample preparations for the determination of lupine were according to the instructions of the Ridascreen ELISA kit (Anon, 2021). Preparation stages of breakfast cereal, oatmeals, biscuit, and chocolate samples for analysis generally consist of homogenization, extraction, and centrifugation processes. According to the kit procedure, 1 g of the homogenized sample was transferred to centrifuge tubes and 20 ml of diluted extraction buffer heated to 60 °C was added, and extraction was carried out at 60 °C for 10 min with thorough mixing. After the prepared mixture was cooled, it was centrifuged at 2,500 x g for 10 min (Sigma 2-16 KL, Germany) and the supernatant layer was collected. One hundred microliters of the standard solution and each of the samples were added to the wells of the microplates and mixed gently and incubated for 10 min at 20 - 25 °C. At the end of the incubation, the liquid was poured from the wells and washed four times with the washing buffer. After this stage, one hundred microliters of the diluted conjugate solution were added to each well and gently mixed by shaking the microplate by hand. It was incubated for 10 minutes at room temperature (20 - 25 °C) and then the same washing process as previously mentioned was applied. One hundred microliters of the substrate/chromogen solution were added to each well, the microplate was mixed gently by shaking by hand and then incubated for 10 minutes at room temperature (20 - 25 °C) in the dark. In the last step, 100 µL of stop solution was added to each well and was read absorbance at 450 nm in ELISA plate reader (SpectraMax i3x molecular Devices, Germany). Lupine concentrations were calculated through the guidelines of the Ridascreen kit (R-Biopharm AG). The measurement results made in the samples and standards taken for each experiment were calculated in the Rida®Soft Win program. The detection limit (LOD) and the quantification limit (LOQ) of lupine protein were reported as 0.7 mg kg⁻¹ and 1 mg kg⁻¹, respectively.

Statistical Analyses
Descriptive statistical analyzes in which the mean, standard error, minimum and maximum values were determined in the analyzed food samples were made in SPSS 16 version statistical program. An independent sample t-test was used to evaluate the inter-firm differences of the chocolate samples, as well as to compare the biscuit and chocolate groups (Daniel, 1991).

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RESULTS AND DISCUSSIONS

In the present study, breakfast cereals (A, B, C and D), oatmeals (E and F), biscuits (G, H, I, J and K), and chocolates (L, M and N) from different firms were obtained from local markets in Ankara province. The presence of lupine was determined in a total of 90 packaged food samples by sandwich ELISA. It is stated in the label information of some of these products that they contain lupine or may contain traces of lupine. In other analyzed food samples, there is no information about the presence of lupine. The distribution of lupine in food samples is given in Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Firms</th>
<th>Total (positive) sample</th>
<th>Distribution (mg kg⁻¹)</th>
<th>Label information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1.0</td>
<td>1.00 – 1.50</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>A</td>
<td>10 (0)</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4 (0)</td>
<td>4</td>
<td>-</td>
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<tr>
<td></td>
<td>C</td>
<td>10 (0)</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>7 (0)</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Oatmeals</td>
<td>E</td>
<td>6 (0)</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5 (0)</td>
<td>5</td>
<td>-</td>
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<tr>
<td></td>
<td>G</td>
<td>10 (1)</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>5 (1)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Biscuits</td>
<td>I</td>
<td>5 (3)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>8 (0)</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>5 (0)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Chocolates</td>
<td>L</td>
<td>5 (4)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5 (2)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>5 (0)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>90 (11)</td>
<td>79</td>
<td>4</td>
</tr>
</tbody>
</table>

The presence of lupine was detected in 11 (12.22%) of analyzed samples. It was not detected in breakfast cereal and oatmeals. Lupine was found in some biscuit and chocolate samples. The minimum and maximum values of lupine of these samples varied between not detected (nd) and 2.95 mg kg⁻¹, respectively. The mean levels (± standard error - S.E) of positive lupine of biscuits and chocolate samples were found to be 2.53±0.18 mg kg⁻¹ and 2.04±0.16 mg kg⁻¹, respectively. The mean lupine value of 11 biscuit and chocolate samples found to have lupine was 1.81±0.13 mg kg⁻¹. The lupine values (mean ± SE) of the L and M firms of the chocolate samples were determined as 2.23±0.20 mg kg⁻¹ and 1.67±0.15 mg kg⁻¹, respectively. The presence of lupine could not be detected in the samples of the N firm in the analyzed chocolates. In biscuits, the presence of lupine could not be detected in the samples of J and K firms. Lupine values (mean ± SE) of the biscuit samples from G, H, I firms are 1.95±0.87 mg kg⁻¹, 1.18±0.13 mg kg⁻¹, and 1.51±0.16 mg kg⁻¹, respectively. The minimum and maximum lupine values of the biscuit samples ranged from nd to 1.95 mg kg⁻¹, respectively. As a result of the statistical comparison of the mean lupine values of the biscuits with the lupine values of the chocolate samples, the difference between the product groups was found to be significant (p<0.05). As a result of the statistical comparison made with the average lupine values of the companies where positive values were obtained in the chocolate samples, the difference between the product groups was not found significant (p>0.05). When food labels are examined; It has been stated that lupine can be found on the labels of D firm samples in the breakfast cereal product group, the E firm samples in the oatmeal product group, and the samples belonging to the G firm in the biscuit product group. Among the products and firms mentioned here, the presence of lupine was detected in only one product of G firm, as indicated in the label information. However, lupine could not be detected in other products and firms whose label information stated that lupine could be found. Although not specified in the label information, lupine was detected in a total of 10 samples, 4 in biscuits and 6 in chocolates. In this case, it is thought that the differences in the label information may be caused by the cross-contamination in the production technology of the companies.

Turkish Food Codex (TFC) Food Labeling and Consumer Information Regulation (TFC, 2017) has been prepared taking into account the European Parliament and Council Regulation No, 1169/2011 (Regulation EU, 2011). According to the TFC Food Labeling and Consumer Information Regulation, a total of certain substances or products causing allergies or intolerances including lupine and lupine components, must be stated on the labels of all foods supplied to the final consumer.

To our knowledge, the presence of lupine in food products included has not been investigated in Turkey. Therefore, it will not be possible to make a comparison for our country. It is thought that this study will guide future research as the first study within our knowledge that indicates the importance of analytical control of lupine, also known as a hidden allergen, in our country. It is seen that the studies carried out abroad are mostly method development studies related to lupine.
detection. Ecker and Markl (2012) developed a sandwich ELISA for lupine analysis in foods. They stated that lupine was below the detection limit of ELISA in 8 different food samples collected from the markets, such as cookies, rusks, crispbread, fresh bread, noodles, tofu, vegetarian patties, and vegetarian type patties that could potentially contain lupine. Mattarozzi et al. (2012) developed a method for the detection of multiple allergens and analyzed lupine in a total of 5 pasta and 5 biscuit samples made directly from lupine flour, obtained from local stores and labeled as "may contain traces of lupine". They stated that in these food products, with the LC–ESI–SRM–MS method, the lupine amounts were below the detection limit in 2 pasta and 1 biscuit samples, which were stated to contain trace amounts of lupine on their labels, and they detected lupine in 2 biscuits as indicated in the label information. Ecker et al. (2013) investigated the presence of lupine by comparing ELISA and Real-time polymerase chain reaction (Real-Time PCR) methods in 43 commercial food samples taken from local markets in Austria. The researchers stated that they detected lupine in 15 of 25 food samples whose label information stated the amount of lupine, and that lupine was not detected in one food sample that was stated as lupine-free in the label information. On the other hand, in 8 food samples, which were stated to contain trace amounts of lupine on the label, lupine was detected between below the LOD value (10 ppm) and 180 ppm by the sandwich ELISA method, and the presence of lupine was lower than LOD value in 9 food samples without label information about the lupine content. Villa et al. (2018) investigated the presence of lupine in 26 food products (such as cookies, cupcakes, cakes, wafers and patisserie products) obtained from markets in Portugal by Real-Time PCR. It was stated in the label information of these products that they do not contain lupine, contain lupine, or may contain traces of lupine. They reported that there was lupine in 2 of 14 food samples that were stated to contain traces of lupine on the label and that the lupine content was estimated to be between 4.12% and 22.9% in 5 food samples that were stated to contain lupine on the label. In our study, similar to these studies, lupine could not be detected in some products on the label of which it was stated that they might contain traces of lupine as an allergen warning. It is thought that this may be due to the precautionary labeling practices of the companies or the lupine content of these foods being too low for the method to detect.

CONCLUSIONS

In the present study, it was aimed to investigate the presence of lupine by sandwich ELISA in some packaged food products including breakfast cereal, oatmeal, chocolate, and biscuits collected from Ankara, Turkey. Lupine was detected in 11 (12.22%) of 90 food samples. There was no information about the presence of lupine on the labels of 10 lupine detected food samples. The presence of allergic compounds in foods and the absence of these allergen compounds on the label reduce the safety of the food item and adversely affect human health and cause serious health problems, to shed light on future studies, to raise awareness about lupine among producers and consumers, and especially to emphasize the value of food label information.

Author Contributions: N.G. Master thesis, conceived, designed and performed the analysis, collected the data, wrote the paper; B.E.D. Conceived, designed and performed the analysis, wrote the paper; B.D. Performed the analysis, collected the data and wrote the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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