

Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Lupine Residues in Foods

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ABSTRACT: Lupine has been increasingly used in food applications due to its high nutritional value and excellent functional properties. However, lupine provokes allergic reactions in susceptible individuals. The presence of undeclared lupine residues in foods can pose a serious health risk to lupine-allergic individuals. Therefore, the objective of this research was to develop a sandwich-type ELISA for the detection of lupine residues in foods. Lupine flour derived from *Lupinus albus* was used to immunize 3 rabbits and a sheep. Pooled lupine-specific antibodies were partially purified from the sera by ammonium sulfate precipitation. A sandwich lupine ELISA with a limit of quantification (LOQ) of 1 ppm was developed by utilizing the rabbit antisera as the capture reagent and the sheep antiserum as the detector reagent. The binding of the antigen-antibody complex was visualized by the addition of commercial rabbit antiserum IgG antibody labeled with alkaline phosphatase with subsequent addition of *p*-nitrophenyl phosphate substrate to produce a colored product for quantification. Minor cross-reactivity was observed with soy (*Glycine max*) and black bean (*Castanospermum australe*). The performance of the lupine ELISA was evaluated in reference food standards (beef frankfurter and apple cinnamon muffin) and laboratory-prepared cooked frankfurters and corn muffins. The mean percent recovery for lupine spiked-frankfurters and corn muffins were $108.4\% \pm 8.8\%$ and $103.1\% \pm 11.5\%$, respectively. The sandwich-type lupine ELISA developed in this study provides food manufacturers and regulatory agencies with an effective analytical tool to detect and quantify lupine residues in processed foods.

Keywords: allergen control, ELISA, immunoassay, lupine, lupine allergy

Introduction

Lupine is a legume belonging to the Fabaceae family, which also includes other edible legumes such as soybean, pea, lentil, and peanut (Kurlovich and others 2002). The consumption of lupine has increased notably, especially in certain European countries and Australia, following the introduction of lupine flour as a commercial food ingredient in the mid-1990s (Peeters and others 2007a). The most common lupine species grown for human consumption are *Lupinus albus* (white lupine), *Lupinus angustifolius* (blue lupine), *Lupinus luteus* (yellow lupine), and *Lupinus mutabilis* (pearl lupine), (Ballester and others 1984; Kurlovich and others 2002). Lupine has a wide variety of uses in food applications due to the high protein content of lupine seeds that provide both nutritional and functional properties in food products (EFSA 2005). Lupine flour was earmarked as the next major competitor to soybean as a protein source, especially in European countries that do not accept genetically modified crops as human foods (Radcliffe and others 2005; Daniells 2006). In addition, lupine is a gluten-free legume and is used for gluten-free products for individuals suffering from celiac disease (Matheu and others 1999; BfR 2006).

The application of lupine in foods can be limited because of allergenic proteins that can trigger allergic reactions in susceptible individuals (Hefle and others 1994; Smith and others 2004; Holden and others 2005). The prevalence of lupine allergy in the general

population is unknown, but is apparently rising due to the increased availability of lupine products in the market (Holden and others 2005; Peeters and others 2007a). More attention has been recently devoted to lupine allergy because of the severity of the allergic reactions and the potential clinical cross-reactivity with other legumes, particularly peanut. Although no fatalities have been attributed to lupine allergy, several lupine-sensitive individuals have experienced life-threatening anaphylactic shock after the ingestion of lupine-containing products (Matheu and others 1999; Kanny and others 2000; Radcliffe and others 2005; Jappe and others 2007). Lupine has been added to the list of common allergenic foods that require mandatory labeling on foodstuffs sold in the European Union (EFSA 2005).

Lupine-allergic individuals must adhere to a strict avoidance diet (Taylor 2006). However, the successful implementation of an avoidance diet can be challenging due to mislabeling issues and inadvertent contamination during manufacturing processes such as the use of shared equipment, inadequate clean-up procedures, and the use of rework (Poms and Anklam 2006; Taylor and Hefle 2006). Consequently, an analytical method that can accurately detect lupine residues in foods is needed to assist food manufacturers and regulatory agencies to evaluate allergen control strategies, to investigate consumer complaints, and to ensure compliance with labeling regulations.

Tracking allergenic lupine proteins has become an important task for food manufacturers because of the risk of inadvertent contamination and consumer safety concerns (Poms and Anklam 2006). The objective of this research was to develop an ELISA for the detection of lupine residues in a wide range of foods. A sandwich-type ELISA was the method of choice as this methodology is

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sensitive, specific, and has been successfully applied for the quantitative analysis of other commonly allergenic food residues in foods (Hefle and others 1994, 2001; Koppelman and others 1996; Hlywka and others 2000; Poms and others 2004; Lee and others 2008).

Materials and Methods

Polyclonal antisera production

Polyclonal antisera against lupine were produced at Lampire Biological Lab. Inc. (Pipersville, Pa., U.S.A.). Three New Zealand rabbits and a sheep were immunized with lupine flour as the immunogen; the flour was obtained from sweet white lupine seeds (Magenta Sales Ltd., Goostrey, Cheshire, U.K.). The lupine flour immunogen was reconstituted with phosphate buffered saline to 1 mg/mL at the Lampire Biological Lab. Inc. The protocol for production and collection of the lupine antisera in rabbits and sheep was identical to that used previously for antimustard sera (Lee and others 2008). The specificity and titer of the antisera were monitored throughout the immunization period using a noncompetitive ELISA as described (Hefle and others 2001), with modifications (Lee and others 2008). Titer was defined as the log reciprocal dilution of antiserum that gives half-maximal binding in the linear portion of the standard ELISA titration format when 1 µg/mL of lupine immunogen is coated onto the microtiter plate (Hefle and Lambrecht 2004). Antisera with titer values ≥ 10000 were pooled according to species and were partially purified by ammonium sulfate precipitation following the procedure outlined by Harlow and Lane (1988). The protein content of the purified pooled sera were determined by the Lowry method (Lowry and others 1951) and stored at -20°C for use in developing the lupine ELISA.

Standards and samples extractions

Commercial food samples used for the analysis were purchased from local grocery stores. Solid samples were ground to uniform consistency using an Osterizer® blender (Sunbeam Corp., Delray Beach, Fla., U.S.A.) or a commercial food processor (KPF600 Food Processor, Kitchen Aid®, St. Joseph, Mo., U.S.A.). Liquid and oil samples were used as is without any grinding operations.

Lupine flour immunogen, lupine standards, food samples for the cross-reactivity study, the reference food standards and the manufactured model foods were extracted 1:10 (weight/volume) in 0.01 M phosphate buffered saline (PBS; 0.002 M NaH_2PO_4 , 0.008 M Na_2HPO_4 , 0.85% NaCl, 0.02% NaN_3 , pH 7.4) at 140 rpm in a shaking water bath (Julabo SW22, Julabo U.S.A. Inc., Allentown, Pa., U.S.A.) at 60°C for 2 h. Extracts were then centrifuged at $3612 \times g$ (4100 rpm) in a tabletop centrifuge (Sorvall® Legend™ RT, Kendro Lab. Products, Newton, Conn., U.S.A.) at 10°C for 30 min. Protein content of the solutions was determined by the Lowry method (Lowry and others 1951). The clarified solutions were divided into aliquots and stored at -20°C until analyzed.

Lupine standards

Initially, a standard containing 1000 ppm lupine flour immunogen was prepared in PBS. A series of lupine flour standards was prepared by serially diluting the 1000 ppm lupine-spiked PBS at a volume ratio of 1:3 in PBS to achieve levels ranging from 0 to 1000 ppm (1000, 333, 111, 37, 12.3, 4.12, 1.37, 0.457, 0.152, 0.051, 0.017, and 0 ppm lupine flour).

ELISA

The sandwich ELISA was developed according to a published protocol (Hefle and Lambrecht 2004) with minor modifications as described below. Microtiter plates (NUNC-Immuno™

MaxiSorp™ 96-MicroWell™ plates, Nalgel Nunc Intl., Rochester, N.Y.) were coated with 100 µL/well of rabbit antilupine sera at 10 µg/mL in coating buffer (0.015 M Na_2CO_3 , 0.035 M NaHCO_3 , and 0.02% NaN_3 , pH 9.6) and incubated overnight at 4°C . The plates were then blocked with 350 µL/well of blocking buffer consisting of 0.1% gelatin (from porcine skin, 300 bloom, Sigma-Aldrich, St. Louis, Mo., U.S.A.) in PBS for 1 h at 37°C . The bound antigen was detected by adding 100 µL/well of 5 µg/mL sheep antilupine sera prepared in conjugate buffer (PBS containing 1% bovine serum albumin [RIA grade, Sigma Chemical Co.], pH 7.4) and incubated for 1 h at 37°C . The extent of binding of antigen-antibody complex was determined by the addition of commercial rabbit antiserum IgG antibody labeled with alkaline phosphatase, diluted 1:2500 in conjugate buffer for 1 h at 37°C . Binding was visualized with subsequent addition of *p*-nitrophenyl phosphate substrate (Sigma Fast™, Sigma-Aldrich) with incubation time of 30 min at room temperature in the dark. The enzymatic reaction was then stopped by the addition of 1N NaOH, and the color intensity was read immediately at 405 nm using a microplate reader (ELx808 Ultraplate, Bio-tek Instruments Inc., Winooski, Vt., U.S.A.). Standard curves were generated using GraphPad Prism® Software (GraphPad Software Inc., San Diego, Calif., U.S.A.) by plotting the average of triplicate absorbance readings against the $\log_{10}$ concentration of the lupine standards (parts per million).

Cross-reactivity studies

Foods that are botanically related to lupine and food ingredients that are present in processed foods containing lupine were selected for the cross-reactivity studies. After extraction of the food samples, all extracts were diluted in PBS, and then analyzed in the lupine ELISA on a protein content (10, 1, and 0.1 µg/mL) and concentration (1:10, 1:100, 1:1000) basis.

Reference food standards

Since processed meats and bakery goods can be made with lupine flour in their formulation, raw beef frankfurters and baked apple cinnamon muffins were used as reference food standards. Standards were prepared by adding lupine flour in each food matrix to yield a final concentration of 1000 ppm (weight/weight), followed by extraction and clarification as described previously for the samples. A series of standards was then prepared by serially diluting the 1000 ppm lupine-spiked frankfurter and lupine-spiked muffin extracts 1:3 (volume/volume) in corresponding food extracts to obtain levels ranging from 0 to 1000 ppm (1000, 333, 111, 37, 12.3, 4.12, 1.37, 0.457, 0.152, 0.051, 0.017, and 0 ppm lupine flour). The ELISA standard curves prepared from lupine-spiked raw beef frankfurter or lupine-spiked baked apple cinnamon muffin were compared with the lupine ELISA standard curve prepared in PBS.

Model foods preparation

Frankfurter. The basic formulation of the frankfurters consisted of 8% water, 2% salt, 1% sugar, 0.0156% nitrite salt (93.75% sodium chloride with 6.25% sodium nitrite), and appropriate amounts of meat block (90% lean ground beef) to make up a 1.5 kg batch. Cure solution was prepared by dissolving all dry ingredients listed above with water. The cure solution was then added evenly to the meat blocks that were placed in a food cutter (model 84181D, Hobart, Troy, Ohio, U.S.A.) and chopped at high speed for 3 min. The finished batter was immediately stuffed into 50 mL conical tubes (BD Falcon™, Franklin Lakes, N.J., U.S.A.) and was cooked in an 80°C water bath (Julabo SW22, Julabo U.S.A. Inc.) until an internal temperature of 71°C was achieved. Frankfurters were weighed before and after cooking to determine the cooking yield.

The cooked frankfurters were evaluated immediately, and any remainder was stored at -20°C .

Lupine flour at 0, 1, 10, 50, 100, 500, and 1000 ppm (milligrams of lupine flour per kilogram of frankfurter) was introduced independently into the cure solution just prior to the addition to the uncooked meat block. Since the 1, 10, 50, and 100 ppm levels of lupine required minute amounts of lupine flour, a 10000 ppm lupine flour-in-salt mixture (1 g of lupine flour added to 99 g of salt) was prepared and then incorporated into the meat formulation in place of a portion of the salt to achieve the desired levels.

Corn muffins. The ingredients of corn muffin model food included Always Save[®] corn muffin mix (Associated Wholesale Grocers, Inc., Kansas City, Kans., U.S.A.), large eggs, and milk (2% fat). The basic formulation of the corn muffin consisted of 15% egg, 23% milk, and appropriate amounts of lupine-corn muffin mixture to make up a batter batch of 338 g. Lupine flour at 0, 1, 10, 50, 100, 500, and 1000 ppm (milligrams of lupine flour per kilogram of corn muffin) was added independently to the corn muffin mix and stored at room temperature until use. Since the 1, 10, 50, and 100 ppm levels of lupine required minute amounts of lupine, a 1000 ppm lupine flour in Always Save[®] corn muffin mix (0.1 g of lupine flour added to 99.9 g of Always Save[®] corn muffin mix) was prepared and an appropriate amount of the mixture was incorporated into the corn muffin formulation to achieve the desired level.

The mixtures of lupine flour and corn muffin mix were sifted 7 times to ensure that the lupine flour was equally distributed in the corn muffin mix. Three subsamples of each level of lupine in corn muffin mix were taken from different areas; top (A), left (B), and right (C). These samples were extracted 1:10 (w/v) in 0.01 M PBS at 140 rpm in a shaking water bath (Julabo SW22, Julabo U.S.A Inc.) at 60°C for 2 h. The extracted samples were centrifuged at $3612 \times g$ (4100 rpm) for 30 min at 10°C in a tabletop centrifuge (Sorvall[®] Legend[™] RT, Kendro Lab. Products). The clarified supernatant solutions were tested for homogeneity using the lupine ELISA.

Prewheated milk and egg were added together and stirred for 30 s. The mixture of lupine flour and corn muffin mix were sifted 2 more times prior to the addition to egg and milk. The muffin batters were prepared in a bowl by combining all ingredients and were thoroughly mixed in a mixer (KSM150PS Artisan 5-Quart Stand Mixer, Kitchen Aid[®]) under medium speed (speed #4) for 3 min. Each of the 7 levels (0, 1, 10, 50, 100, 500, 1000 ppm or milligrams of lupine flour per kilogram of corn muffin) was made independently starting with the 0 ppm level and continuing with the next lowest level. Between batches, a simple cleaning of the equipment was done using hot and distilled water. The finished batter was immediately transferred into a round muffin cup and put into an oven for 18 min at 400°F . After baking, the muffins were allowed to cool to room temperature. Muffins were weighed before and after baking to determine the cooking yield. The muffins were evaluated fresh, and the remainders were stored at -20°C .

Frankfurter and corn muffin model foods

Each of the frankfurters and corn muffins containing various levels of lupine flour was extracted in duplicate, and each extract was analyzed in triplicate in the lupine ELISA.

Statistical analysis

The differences between the standard curves with various levels of lupine flour spiked into PBS, corn muffin, and beef frankfurter were performed using the *F* test (GraphPad Prism[®] Software).

Results and Discussion

Antibody production

The choice of the immunogen is particularly important in developing an effective ELISA that is capable of detecting trace amounts of lupine residues in complex food matrices. White lupine flour (*Lupinus albus*) was chosen to acquire lupine-specific antibody in immunized animals because this lupine species is the most widely utilized species in food applications especially in Europe where most lupine allergy cases are reported. The analysis of prebleeds demonstrated that these animals did not possess antibodies against lupine flour before immunization (data not shown). Pooled rabbit and sheep antiserum with titers of 9250 ($10^{3.97}$) and 64400 ($10^{4.81}$), respectively was used in developing the lupine ELISA.

ELISA

The lower limit of quantification (LOQ) is defined as the lowest level of lupine that can be detected in the ELISA with acceptable accuracy and precision. The optimized lupine ELISA is a robust and sensitive assay with a LOQ of 1 ppm (Figure 1). The threshold doses that elicit allergic reactions in lupine-allergic patients have not been determined yet. However, several studies have been conducted to examine the minimal eliciting doses in a few patients using oral challenges with foods containing a known amount of lupine (Moneret-Vautrin and others 1999; Crespo and others 2001; Peeters and others 2007). While threshold doses can vary depending on the sensitivity of the individual, the minimal eliciting doses in the most sensitive individual in the challenge trials was 0.5 mg of lupine flour for subjective symptoms (Peeters and others 2008). As 0.5 mg is equal to 10 ppm of lupine (1 ppm = 1 mg/kg of food) in a typical food serving size of 50 g (Taylor and others 2002), the 1 ppm LOQ of the developed sandwich ELISA for lupine is considered appropriate.

Cross-reactivity

Cross-reactivity assessment is necessary when developing an immunoassay for detecting allergenic residues in foods, due to the variety of food ingredients used in the manufacture of packaged food products (Yeung 2006). The specificity of the lupine ELISA was evaluated by testing a total of 131 foods and food ingredients including 15 legumes from the Fabaceae family and 5 legumes from other families. As shown in Table 1, the polyclonal antisera

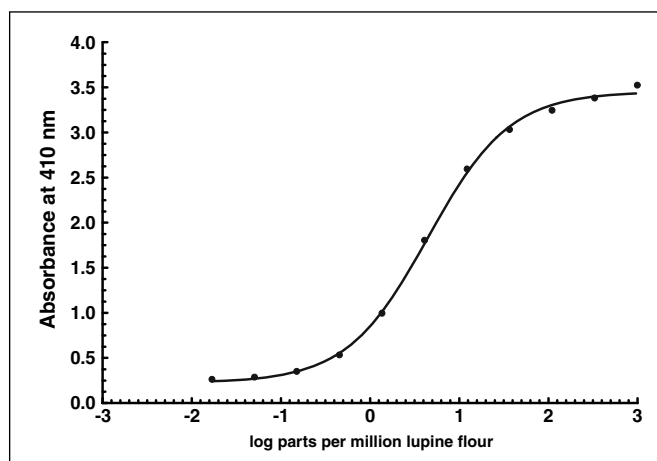


Figure 1—Standard curves obtained with the lupine ELISA. Each data point represents the mean of 9 readings, with standard deviation of < 0.10 absorbance unit.

used in the optimized lupine ELISA were specific for lupine. Of the 131 foods and ingredients, only soy (*Glycine max*) and black bean (*Castanospermum australe*) showed weak cross-reactivity, which were equivalent to approximately 1 ppm of lupine when concentrated extracts were tested. The minor cross-reactivity of lupine and black bean and soy is likely due to the presence of highly homologous seed storage proteins in legume seeds,

such as 7S globulins (vicilins) and 11S globulins (legumins). The low level of cross-reactivity will not be detrimental to the assay performance and would only be a concern if highly concentrated soy- or black bean-based foods were tested for lupine residues.

Reference food standards

A preliminary study was performed with reference food standards (beef frankfurter and apple cinnamon muffin) to evaluate any possible matrix interference that could affect the lupine ELISA assay sensitivity and to determine the suitable food matrices for the production of manufactured model foods. Figure 2 compares the standard curves using the standards in 0.01 M PBS and in both frankfurter and muffin matrices. The frankfurter matrix, which was composed of beef, sugar, salt, and nitrite did not substantially affect the sensitivity of the lupine ELISA. The slope of the PBS standard curve and frankfurter standard curve was not significantly different ($P > 0.3$). The frankfurter standard curve shifted slightly to the left, which results in a slight increase in the sensitivity level of the ELISA. No observable differences were noted between the ELISA standard curve generated in the muffin matrix and in 0.01 M PBS. The slope of the standard curves were not significantly different ($P > 0.3$), indicating that the sensitivity of lupine ELISA was not affected by the muffin matrix, which was composed of wheat flour, egg, milk, sugar, vegetable oil, baking powder, and salt.

Model foods

The preliminary experiments with reference food standards demonstrated that the lupine ELISA is capable of detecting lupine proteins in selected food matrices without interference. Lupine flour is used as an ingredient in processed meat products in some countries. Therefore, the ability of the sandwich ELISA to accurately quantify the levels of lupine in such foods is critically important. Thus, frankfurter was chosen as a model food and was produced under conditions that closely resemble the food industry manufacturing process to evaluate the performance of the assay in terms of extraction efficiency, matrix interference, and true recovery of the lupine residues after the lupine was incorporated in the initial formulation (as opposed to spiking) and subjected to processing conditions. Spiking experiments do not

Table 1—List of foods and food ingredients showing no cross-reactivity in the lupine ELISA.

Fabaceae	Nuts/seeds	Functional ingredients
Alfalfa sprout	Almond	Agar
Black gram	Cashew	Baking soda
Chickpea	Hazelnut	Chocolate (milk)
Cowpea	Macadamia	Coconut (flakes)
Fava bean	Nutmeg	Coffee (seed)
Kidney bean	Pecan	Coffee (powder)
Lentil	Pine nut	Guar gum
Lima bean	Pistachios	Lemon juice
Mungbean	Poppy seeds	Lime juice
Pea (green)	Sesame seed	Peanut butter
Pea (yellow, split)	Walnut	Sodium alginate
Peanut		Soy isolate
Snow pea		Soy lecithin
	Sugars/	Soy sauce (acid
	Sweeteners	hydrolyzed)
Other legumes	Cane syrup	Soy sauce (naturally
Great northern bean	Corn syrup (high	fermented)
Green bean	fructose)	Soymilk
Mayocoba bean	Corn syrup (light)	Tapioca
Navy bean (pea	Corn syrup (solids)	Vanilla extract
beans)	Maltodextrin	Vinegar (apple cider,
Pink bean	Molasses (dark)	balsamic)
	Molasses (light)	Vinegar (distilled)
	Sorbitol	Xanthan gum
Dairy ingredients		Yeast brewer
Buttermilk powder	Meats	
Casein	Ground pork	Preservatives
Non-fat dry milk	Raw turkey	Citric acid
(NFDN)	Raw hamburger	Nitrite (Sodium)
Shortening		
Whey powder	Herbs and spices	
Yogurt	Allspice (ground)	Fruits
	Basil leaves	Banana
Cereals (flours,	Cinnamon	Blueberry (canned)
starches, and	Cloves	Blueberry (fresh)
meal)	Cumin (ground)	Blueberry (dried)
Buckwheat flour	Garlic (minced	
Carob flour	dried)	Oils
Corn	Garlic powder	Canola oil
Corn flour	Ginger powder	Corn oil
Corn meal (yellow)	Lemon and pepper	Peanut oil
Cornstarch	MSG	Soybean oil
Malt (barley)	Mustard (ground)	Sunflower oil
Oat bran	Onion (minced	Vegetables oil
Whole oat flour	dried)	
Oats	Onion (powder)	Eggs
Potato	Oregano	Egg white
Potato starch	Paprika	Spray dried egg white
Rice (brown)	Parsley	Spray dried whole egg
Rice (white)	Pepper (black)	Whole egg
Rice flour	Pepper (white)	
Rollled whole	Salt	
wheat	Thyme	
Rye		
Rye flour		
Sorghum seed		
Soy flour		
Wheat		
Wheat bran		
Wheat flour		
Wheat gluten		
Whole wheat flour		

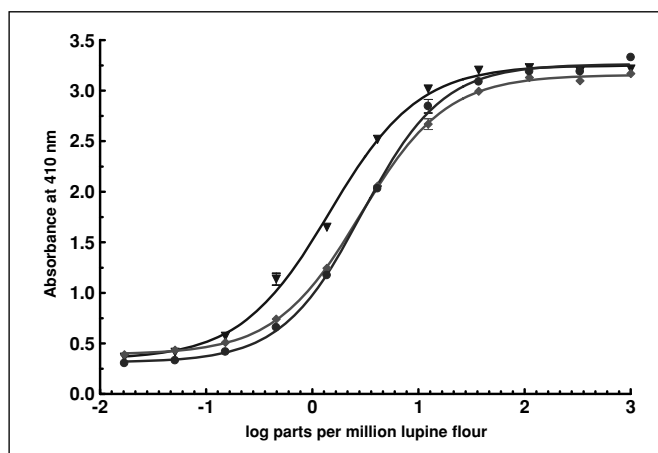


Figure 2—Standard curves of 1000 ppm lupine flour-spiked PBS (●), apple cinnamon muffin (◆), and beef frankfurter (▼) in the lupine ELISA. Each data point represents the mean of 9 readings, with standard deviation of < 0.08 absorbance unit.

Table 2—Recovery and percent recovery of lupine residues from the manufactured model cooked frankfurters, as determined by the lupine ELISA.^a

Spike levels	Levels detected in ELISA (ppm ^b lupine)				Average ^c (ppm lupine)	% Recovery ^c
	Extract A		Extract B			
	Trial 1	Trial 2	Trial 1	Trial 2		
0 ppm	BLQ ^d	BLQ	BLQ	BLQ	NA ^e	NA
1 ppm	1.3	1.1	1.1	1.2	1.2 ± 0.1	117 ± 9.6
10 ppm	11.9	11.6	11.3	12.3	11.8 ± 0.4	118 ± 4.3
50 ppm	49.6	35.9	50.8	61.3	49.4 ± 10.4	98.8 ± 20.8
100 ppm	99.6	83.1	102	104	97.0 ± 9.4	97.0 ± 9.4
500 ppm	476	507	549	521	513 ± 30.3	103 ± 6.1
1000 ppm	1130	1190	1160	118	1170 ± 27.2	117 ± 2.7

^aStandard used for the generation of standard curve in ELISA was cooked frankfurter extracts containing 1000 ppm lupine flour.^bppm = parts per million (milligrams of lupine per kilogram).^cData are mean ± SD.^dBLQ = Below limit of quantification, < 1 ppm.^eNA = Not applicable.**Table 3—Recovery and percent recovery of lupine residues from the manufactured model corn muffins, as determined by the lupine ELISA.^a**

Spike levels	Levels detected in ELISA (ppm ^b lupine)				Average ^c (ppm lupine)	% Recovery ^c
	Extract A		Extract B			
	Trial 1	Trial 2	Trial 1	Trial 2		
0 ppm	BLQ ^d	BLQ	BLQ	BLQ	NA ^e	NA
1 ppm	1.1	1.2	1.0	1.0	1.1 ± 0.1	108 ± 9.6
10 ppm	8.3	9.1	9.1	9.7	9.0 ± 0.6	90.5 ± 5.7
50 ppm	44.6	56.5	47.8	58.6	51.9 ± 6.7	104 ± 13.5
100 ppm	96.9	109	103	111	105 ± 6.3	105 ± 6.3
500 ppm	463	564	409	443	470 ± 67	94.0 ± 13.4
1000 ppm	997	1330	1000	1380	1180 ± 207	118 ± 20.7

^aStandard used for the generation of standard curve in ELISA was corn muffin extracts containing 1000 ppm lupine flour.^bppm = parts per million (milligrams of lupine per kilogram).^cData are mean ± SD.^dBLQ = Below limit of quantification, < 1 ppm.^eNA = Not applicable.

constitute an appropriate validation because processing can affect the extraction efficiency of an analyte from processed foods (Hefle and Koppelman 2006). The manufactured model foods, produced according to industrial norms, are necessary to assess the extraction efficiency and the applicability of the assay in complex, processed food matrices. In addition, the effects of processing on assay sensitivity and reproducibility are also evaluated through the recovery experiments in manufactured model foods (Immer 2006).

The mean percent recovery for cooked frankfurters and corn muffin were 108% ± 8.8% (Table 2) and 103% ± 11.5% (Table 3), respectively. The excellent recoveries of lupine residues in both model foods indicate that the extraction method was efficient and the antibodies in the lupine ELISA were able to recognize the lupine proteins after processing treatments.

Conclusions

The sandwich-type lupine ELISA developed in this study is an important analytical tool for food manufacturers and regulatory agencies in sanitation validation and the evaluation of allergen control strategies. Although the lupine ELISA has been validated in 2 reference food standards and 2 manufactured model foods, an interlaboratory validation study should be performed to define the statistical parameters of the assay. Additional manufactured model foods should be assessed, especially as uses for lupine in a variety of food products continue to expand. In the future, the developed lupine ELISA, together with the food labeling regulation, could ultimately lead to improvements in allergen management in the food industry.

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