

Accepted Manuscript

Analytical Methods

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PII: S0308-8146(13)01253-3

DOI: <http://dx.doi.org/10.1016/j.foodchem.2013.09.013>

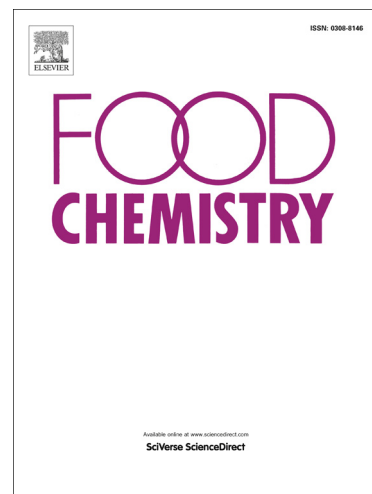
Reference: FOCH 14639

To appear in: *Food Chemistry*

Received Date: 10 June 2011

Revised Date: 1 September 2013

Accepted Date: 4 September 2013



Please cite this article as: Wang, H., Li, G., Wu, Y., Yuan, F., Chen, Y., Development of an indirect competitive immunoassay for walnut protein component in food, *Food Chemistry* (2013), doi: <http://dx.doi.org/10.1016/j.foodchem.2013.09.013>

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Running title: **Development indirect competitive immunoassay walnut
protein component food**

**Title: Development of an indirect competitive immunoassay for walnut
protein component in food**

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19 Abstract

20 Among food allergens, walnut is a frequent cause of adverse food reactions in
21 allergic patients. In this study, the walnut allergen protein 2S albumin precursor
22 (*Jug r 1*) cDNA was synthesized and cloned into the pGEX-6P-1 expression
23 vector. The recombinant plasmids were transformed into *Escherichia coli* (*E.coli*)
24 BL21(DE3) pLys for expression of protein *Jug r 1*. Polyclonal antibodies were
25 prepared against the expressed purified *Jug r 1* protein. An indirect competitive
26 enzyme-linked immunosorbent assay (ELISA) for the detection and
27 quantification of walnut soluble proteins in processed foods was developed
28 using the prepared polyclonal antibodies. The developed ELISA had a high
29 specificity, walnut protein standard solution at 2.2 ng/mL[inhibition
30 concentration (IC₈₀) of the competitive test] was clearly identified by the
31 ELISA. The mean recoveries ranged from 86 % to 112 %. The coefficient of
32 variation (CV) for the 4 model foods was 6.4 %-8.7 %.

33 **Keywords:** Walnut, Allergen, Indirect competitive, ELISA

34 Introduction

35 IgE-mediated type I hypersensitivity to foods affects approximately 1 % to 3
36 % of the adult population and up to 8 % of the pediatric population (Zeiger,
37 1990). Allergies to tree nut (and peanut) generally begin in childhood, but unlike
38 allergies to a number of other foods, persist throughout life (Ewan, 1996; Crespo,
39 & Rodriguez, 2003). However, it is likely, though not yet proven, that some
40 patients with an early onset of milder forms of allergy will become tolerant of
41 tree nuts after a period of avoidance, analogous to what has recently been shown
42 for a minority of peanut-allergic patients (Sicherer, 2000). The seriousness of
43 tree nut allergy is indicated by the fact that all the fatal allergic reactions to
44 foods for individuals over the age of 6 reported to a US national registry have
45 been caused by either peanuts or tree nuts (Bock, Munoz-Furlong, & Sampson,
46 2001).

47 In the US, allergic patients to walnut (34 %) are more than allergic patients
48 to any other tree nut (Sicherer, Furlong, & Munoz-Furlong, 2001). Of 54
49 pediatric tree nut-allergic subjects in one study, 26 % were confirmed to be
50 reactive to walnut seed protein extract (Sicherer, Burks, & Sampson, 1998).
51 Although the black walnut species, *Juglans nigra* and *Juglans californica*,
52 are fairly common in North American, they are not eaten. Consequently, most
53 walnut-allergic patients have probably been sensitized to the commercially
54 available and widely consumed English walnut (*Juglans regia*).

55 Several English walnut allergens have been identified. The first to be

identified was a 2S albumin precursor, Jug r 1, with a molecular mass of 15 kD-16 kD, the mature Jug r 1 allergen consists of a large subunit with a molecular mass about 10 kD-12 kD and a small subunit with a molecular mass about 4 kD-5 kD. The second allergen to be identified was a member to the vicilin family of seed storage proteins (a 7S globulin), Jug r 2, with a molecular mass of 47 kD-48 kD (Teuber, Dandekar, & Peteson, 1998). The third and the fourth allergens were Jug r3 (Asero, Mistrello, & Roncarolo, 2000) and Jug r4 (Teuber, Peteson, & Uratsu, 2003), corresponding to PR-14(LTP) and legumin (11S) respectively. Among these walnuts allergens, Jug r 1 is a major allergen, its allergenicity is retained stably during heat processing (Teuber et al, 1998). Even small amounts of walnut can cause severe reactions, and thus strict management is required to minimize the potential harm. Currently, there are several published methods available for the detection of gene coding for allergens and protein of walnuts in food products, including common PCR (Yano, Sakai, Uchida, 2007), real-time PCR (Brežn'á, Hudecov'á, & Kuchta, 2006) and ELISA (Hirotohi, Yuki, & Haruki, 2008) methods. The PCR methods are specific, but ELISA method based on polyclonal antibodies generated against extracted allergen protein from walnut, it's a small subunit of about 4 kD-5 kD of mature Jug r 1. Extraction of the Jug r 1 allergen protein is arduous and lacks of repetition, so a simple and reliable detection method for hidden walnut is necessary.

In the present study, the *Jug r 1* gene, codify for the walnut allergen protein

Jug r 1 was synthesized and cloned into the pGEX-6P-1 expression vector. The recombinant plasmid was transformed into *E.coli* BL21(DE3) pLys for expression. Polyclonal antibodies were prepared after the expressed protein Jug r 1 being purified. An indirect competitive ELISA was developed for the detection and quantification of walnut soluble proteins in processed foods using the prepared polyclonal antibodies.

Materials and methods

Nut and other samples All samples, including Walnut (*Juglans regia*), Hickory (*Carya cathayensis* Sarg), Pecan (*Carya illinoensis* koch), Pistachio nut (*Pistacia vera*), Cashew (*Artocarpus heterophyllus* lam), Peanut (*Arachis hypogae*), Brazil nut (*Bertholletia excelsa*), Hazelnut (*Corylus spp*), Almond (*Hodgsonia macrocarpa*), Korean Pine seed (*Pinus koraiensis siebet zucc*), Sunflower seeds (*Fructus helianthi annui*), Sesame (*Sesamum indicum*), Pumpkinseed (*Cucurbita pepo*), Rice (*Oryza sativa*), Corn (*Cornū*), Soy (*Glycine max*), Common wheat (*Triticum aestivum*) powder, Milk (*Lac vaccinum*), Beef (Bov), Mutton(Mult), Chicken (*Pullus*), Pork(*Porcus*) and Duck (*Anas domestica linnaeus*), were bought from local market. Excepting milk, for roasted samples, the samples were roasted at 120°C for 15 min using an oven (MT10KC-AW, midea Croup, China)

Reagents and instrumentation Taq DNA polymerase(5 U/μL), T4 DNA Ligase, Restriction enzyme *EcoR I* and *XhoI I*, MiniBEST plasmid purification kit ver.2.0 and Agarose gel DNA purification kit ver.2.0 were purchased from

Takara Co.(China); pGEX-6p-1 plasmid, *E.coli* BL21 (DE3) Plys, MagneGSTTM protein purification system, 3,3',5,5'-Tetramethylbenzidine (TMB) Substrate solutions and 0.22 μ m Nitrocellulose membranes were purchased from Promega Co.(USA); BCA protein assay kit was purchased from Novagen Co.(USA); Mini-vertical electrophoresis unit and Semi-dry Transfer cell were used in this study. Optical densities of the wells were measured with a microplate reader with KC3 software, a 450-nm sample filter was used for reading optical density.

Buffers Sample buffer [pH6.8], consisted of 50 mM Tris, 10 % glycerol, 2 % sodium dodecylsulfate, 100 mM dithiothreitol, and 0.0025 % bromophenol blue. Extraction buffer [pH 7.4], consisted of 1.0 M NaCl, 0.1 M NaH₂PO₄, 0.1 % (v/v) Tween 20, 0.5 % (v/v) Triton X-100. Blocking buffer consisted of PBS, 0.1 % (v/v) Tween 20 and 5 % skimmed milk (w/v). Dilution buffer consisted of 0.5 % (w/v) skimmed milk and 0.1 % (v/v) Tween 20 in PBS. Plate coating buffer [pH 9.6], consisted of 13 mM Na₂CO₃ and 35 mM NaHCO₃. Stop solution consisted of 2 M sulphuric acid.

Synthesization of nucleotide sequence and cloning for Jug r 1 In order to express the allergen protein Jug r 1 of walnut, a open reading frame of *Jug r 1* nucleotide sequence (Genbank accession number u66866) was selected. For later ease in cloning and expression, the restriction enzyme *EcoR I* site and original codon ATG were added into the 5'-end of the nucleotide sequence, the restriction enzyme *XhoI I* site was added into the 3'-end of the nucleotide sequence. The whole cDNA sequence of 432 bp was synthesized, then the

cDNA sequence was cloned into the pGEX-6P-1 plasmid expression vector, and the recombinant pGEX-6P-1 plasmid expression vector was identified by sequencing. The recombinant pGEX-6P-1 plasmid expression vector will result in the synthesis of foreign proteins as a fusion product.

Expression of fusion protein In order to get the recombinant fusion protein Jug r 1(rJug r 1), the recombinant pGEX-6P-1 plasmids were transformed into protease-deficient *E.coli* BL21(DE3) Plys competent cells and grown in Luria-Bertani broth with ampicillin at 37°C, cell growth was photometrically monitored to reach an optical density of 0.8 at 600 nm wavelength. Protein synthesis was induced by adding IPTG, IPTG was added to a final concentration of 1.0 mM, and incubation was continued at 37°C. Subsequently, cells were harvested after 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, cells were centrifuged and resuspended in sample buffer. In the same way, the blank pGEX-6P-1 plasmids were also transformed into protease-deficient *E.coli* BL21(DE3) Plys as blank control, cells were centrifuged and resuspended in sample buffer after 6 h. Samples were boiled for 5 min in sample buffer, SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out on a mini-vertical electrophoresis unit at a constant current of 20 mA and 15% SDS-polyacrylamide laemmli gel were used. Finally, the expressed proteins were analysed by coomassie brilliant blue staining.

Isolation and purification of fusion protein Fusion proteins rJug r 1 was isolated and purified following the protocol by Frangioni and Neel (Frangioni J

V, & Neel B G, 1993). Briefly, transformed protease-deficient *E.coli* BL21 (DE3) Plys was grown in Luria-Bertani broth with ampicillin at 37°C until the optical density reached 0.6-0.9 at 600 nm. IPTG was added to a final concentration of 1.0 mM, and incubation was continued at 37°C. After 6 h, cells were centrifuged and resuspended in STE buffer. Lysozyme was added to 100 µg/mL and incubated on ice for 15 min. Then dithiothreitol was added to a final concentration of 5 mM. The bacteria were lysed by the addition of N-laurylsarcosine to 1.5 % and then sonicated for 1 min on ice. The lysate was centrifuged at 10,000 g at 4°C for 5 min. The supernatant was removed, Triton X-100 was added to 2 % and vortexed, and the fusion proteins were isolated by the MagneGST™ Protein Purification System.

Production of polyclonal antibodies and western blot identification The rJug r 1 protein solution set at 1 mg/mL was combined with Freund's complete and incomplete adjuvant for using immunization of rabbits (Yeung, Newsome, & Abbott, 2000). Two rabbits were immunized against the rJug r 1 protein. Trial bleeds regularly monitored by ELISA for their titer and the lowest inhibition concentration corresponding to antibody-antigen binding. Then whole blood was collected, and the serum was separated. In order to get the antibodies to specific Jug r 1 protein, the GST Glutathione Sepharose 4B and Jug r 1 protein Glutathione Sepharose 4B were prepared respectively, then polyclonal antibodies to rJug r 1 protein were passed through the two Purification column two times respectively. To determine the reactivity of the antibody to jug r 1

with the expressed protein and the native peptides derived from the Jug r 1 precursor protein, western blot with the antibody to jug r 1 was performed. For western blot, after SDS-PAGE of samples, the protein were transferred to 0.22 μ m nitrocellulose membranes 1.5 h at 0.68 mA /cm² by Semi-dry Tranfer cell. The blots were blocked in blocking buffer for 2 h at room temperature(or overnight at 4°C), and then incubated for 1 h at room temperature in the antibodies to Jug r 1 protein diluted 1:100 in dilution buffer. The next step, strips were washed for 10 min three times in washing buffer at room temperature and incubated with goat anti-rabbit IgG labeled with the HRP for 1 h at room temperature. Finally, the strips were washed three times with washing buffer at room temperature, every time for 10 min, then the TMB substrate solution was added onto strips and the reaction was stopped by the addition of PBS.

Preparation of fine powder for walnut and other samples Raw and roasted Walnut, Hickory, Pecan, Pistachio nut, Cashew, Peanut, Brazil nut, Hazelnut, Almond, Korean Pine seed, Sunflower seeds, Sesame, Pumpkinseed, Beef (Bov), Mutton(Mult), Chicken (Pullus), Pork(Porcus) and Duck (Anas domestica linnaeus) were chopped in a blender, then defatted by acetone, filtered, and air-dried at room temperature overnight. Finally, the defatted materials were ground to fine powder in a mill. Raw and roasted Rice, Corn, Soy were ground to fine powder in a mill directly.

Protein extraction of walnut and other samples Excepting milk, the fine powder sample amounts of 10 g was weighed into 250 mL triangular flask, the

extraction buffer was added into each sample at 100 mL/10 g. The samples were homogenized and shaken vigorously for 1h in a heated water bath at 45°C. Each sample was centrifuged at 3,000 rpm for 5 min and the supernatant was collected and centrifuged at 20,000 g for 30 min at 4°C. Finally, the extract was filtered through filter paper and protein concentration of all solutions(including milk) was checked with BCA protein assay kit. Aliquots of these were stored at -20°C if not used immediately.

Immunoassay The ELISA test was based on an indirect competitive configuration. 96-well plate was coated with walnut protein extract according to a previously described procedure (Ben Rejeb, Fischer-Durand, & Martel, 1998). A microtiter plate was coated at 4°C overnight with the prepared walnut protein solution (100 µL of 31 ng/mL walnut protein in plate coating buffer). After wash five times with washing buffer, the plate was blocked at 37°C for 1 h with the blocking buffer. After washing five times with washing buffer, a 100 µL of diluted standard or sample and 100 µL of 1:160 diluted antibodies (2-fold more concentrated than the final antibodies concentration on the plate) were added into 96-well plate respectively, then incubated at 37°C for 1 h. After washing five times with washing buffer, 100 µL of 1/2500 diluted goat anti-rabbit IgG labeled with the HRP was added to each well, then incubated at 37°C for 1 h. After washing five times with washing buffer, 100 µL substrate solutions was added, the color development was stopped after 10 min by adding 50 µL 2 M H₂SO₄ and the ODs were read within 10 min at 450 nm. The calibration curve

consisted of the relative OD response given by a blank and 7 concentrations walnut protein standard diluted with diluent buffer (0.9 ng/mL, 1.8 ng/mL, 3.7 ng/mL, 7.5 ng/mL, 15 ng/mL, 31 ng/mL, 62.5 ng/mL).

If samples contained higher concentrations of walnut, then larger dilutions were used. For unknown samples, a series of dilutions were run.

According to the test conditions, a walnut protein solution set 1 ng/mL as a final concentration on the plate would give a response equivalent to that of a sample containing 0.2 μ g/g walnut.

The examination of the raw and roasted walnut We examined the walnut soluble protein amounts from raw and roasted walnut using the developed ELISA for the values LOQ after six experiments.

Cross-Reactivity to other samples The cross-reactivity to other samples was investigated using 22 kinds of nut, other plant, meat product and dairy samples. We examined the soluble protein amounts from raw samples using the developed ELISA for the values after three experiments.

The recovery The raw walnut powder was mixed with 4 kinds of ingredients(including Common wheat powder, Rice powder, Corn powder, Soy powder), water and baked at 180°C for 30 min to prepare the processed food samples containing the walnut protein at 10 μ g/g (walnut soluble protein weight/food weight), namely 2 milligrams of the walnut powder was added into 1 kg of food ingredient.

Results and discussion

Expression of Jug r 1 and antibodies identification by western blot The recombinant pGEX-6P-1-jug r 1 plasmids were transformed into *E.coli* BL21(DE3) pLys for expression, the bacteria sediment was collected after exposure to IPTG for 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, the protein was analyzed by SDS-PAGE and the optimum time of protein expression was determined. The SDS-PAGE results showed that the walnut allergen protein jug r 1 was expressed efficiently in fusion protein form, the rJug r 1 fusion protein was detected in the stained gel as a single band at approximately 42 kD (16 kD Jug r 1 precursor protein fused to 26 kD GST tag), the expression level of the fusion protein was highest after exposure to IPTG for 6 h and accounted for approximately 30% of the total cellular proteins (Figure 1A). To determine the reactivity of the antibody to jug r 1 with the expressed protein and the native peptides derived from the Jug r 1 precursor protein, western blot with the antibodies to jug r 1 was performed, the western blot showed that the prepared antibodies appeared to bind 42 kD band for recombinant protein, and to bind 10 kD band for natural protein (Figure 1B). According to the above results, we can conclude that the rJug r 1 fusion protein was active, it can stimulate organism to produce antibodies, the antibodies can bind to the native peptides derived from the Jug r 1 precursor protein, it's a basis for establishing the ELISA method to detect the walnut component in the food.

Construction of indirect competitive ELISA The sensitivity of the indirect immunoassay was optimized by varying the concentration of the coating walnut

protein solution and altering the assay conditions at the first preincubation stage. Lowering the concentration of the coating walnut extract resulted in an improved sensitivity of the assay. The optimum sensitivity was reached with a walnut extract set at 31 ng/mL. Under optimum conditions, the IC₅₀ of the ELISA was 9.7 ng/mL (equivalent to 1.94 µg/g walnut protein in the solid matrix, concentration of walnut protein inhibiting 50 % of the antibodies binding on the plate). The IC₈₀ and IC₉₀, considered respectively as the limit of quantitation (LOQ) and limit of detection (LOD) of the assay could be evaluated at 2.2 ng/mL (equivalent to 0.44 µg/g walnut protein in the solid matrix) and 1.1 ng/mL (equivalent to 0.22 µg/g walnut protein in the solid matrix) respectively. Calibration curves performed with standard walnut protein solutions gave a linear range of 2.2 ng/mL~62.5 ng/mL (figure 2).

The examination of the raw and roasted walnut We examined the walnut soluble protein amounts from raw and roasted walnut using the developed ELISA for the values after six experiments. Consequently, walnut soluble protein amounts of both raw and roasted walnut were not significantly different, the mean values of the LOQ for raw and roasted walnut were 2.2 and 2.1 µg/g, respectively ($P > 0.5$) (Table 1).

Cross-Reactivity to other samples 22 commodities were tested for cross-reactivity by preparing protein extracts. As shown in table 2, the IC₅₀ value of walnut was 1.94 µg/g, the IC₅₀ value of pecan was 985 µg/g, the IC₅₀ values of other samples were greatly more than the IC₅₀ value of the walnut

with the developed ELISA. These results indicated that the developed ELISA had a high specificity.

The recovery To examine the applicability of the established ELISA in processed foods, the recovery study was performed by spiking blank matrixes with walnut protein. As shown in table 3, the mean recoveries for all 4 model samples ranged from 86 % to 112 %. The coefficient of variation (CV) for the 4 model foods was 6.4 % ~8.7 %.

We established the indirect competitive ELISA for the detection of walnut proteins. It is an important examination to verify the utility of the developed ELISA, the developed ELISA has a similar reactivity to the raw and heated walnut. It is also important that the developed ELISA has a high specificity. These results suggest that this method could be specific to the walnut protein. To evaluate the established ELISA for the determination of the walnut soluble proteins in processed foods, a recovery study was examined, the LOD values of the established ELISA for the sample solution was 2.2 ng/mL, equivalent to 0.44 μ g of walnut soluble protein/g of food sample weight. Comparing the ELISA method developed by us with previously published one (Hirotooshi et al., 2008), the LOD values of the ELISA method developed by us was higher than that of the previously published ELISA methods(Hirotooshi, Yuki, & Haruki, 2008), the LOD values of the previously published ELISA methods for the sample solution was 0.39 ng/mL, equivalent to 0.156 μ g of walnut soluble protein/g of food sample weight, this means that the sensitivity of ELISA method developed by us

was not better than that of the previously published ELISA methods(Hirotoshi, Yuki, & Haruki, 2008). But the merits of the ELISA method developed by us lies in: indirect competitive ELISA method is simple and time-saving; Expressed protein in vitro could not only simplify process of antibodies preparation, but also keep antibodies specific and stable, consequently, the method developed by us is specific and stable. Being different from the ELISA method developed by us, the previously published one was based on antibodies generated against the extracted Jug r 1 allergen protein from walnut. Walnut extracts was used for construction of in-vitro ELISA method can result in misleading specific immunological assays. We present indirect evidence to suggest that the Jug r 1 allergen proteins of walnut may not be adequately represented in some commercial preparations. This suggests that the walnut Jug r 1 allergen proteins are difficult to extract in the buffer system used by particular manufacturers.

In conclusion, a cDNA encoding a precursor protein for a major allergen from walnut, the most common tree nut has been cloned and expressed, the antibodies were prepared, a rapid, specific and relatively sensitive immunoassay system was developed to detect walnut soluble proteins in foods. This method has been demonstrated to be suitable for the quantitative measurement of specific walnut proteins in processed foods.

Acknowledgments

This work was supported by National Natural Science Foundation of P. R. of China (31071552), National Key Technologies R&D Program of Ministry of

320 Science and Technology of P. R. of China (2009BADB9B03, 2011BAK10B03)

321 and Special Fund for Quality Inspection Research in the Public Interest (10-46).

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Figure legends

Figure 1. SDS-PAGE analysis of expressed protein and western blot

analysis of polyclonal antibodies. A. SDS-PAGE analysis of expressed

protein. Line 1, cell lysate of bacteria transformed by blank pGEX-6P-1 plasmid

expression vector; Line 2, cell lysate of bacteria transformed by blank

pGEX-6P-1 plasmid expression vector after being induced by IPTG; Line 3, cell

lysate of bacteria transformed by recombinant pGEX-6P-1 plasmid expression

vector; Line 4~10, cell lysate of bacteria transformed by recombinant

pGEX-6P-1 plasmid expression vector after being induced by IPTG for 1h ~7h

respectively; Line 11, protein molecular weight standard. B. Western blot

analysis of polyclonal antibodies. Line1~3 were results of SDS-PAGE: Line1,

cell lysate of bacteria transformed by recombinant pGEX-6P-1 plasmid

expression vector after being induced by IPTG; Line2 and 3, extracted protein

from walnut. Line 4, protein molecular weight standard. Line 5 and 6 were

results of western blot: Line 5, recombinant protein; Line 6, natural protein.

Figure 2. The calibration curves of walnut in indirect competitive ELISA.

Values were determined in duplicate, x axis is Log values of walnut protein

concentration; y axis is competitive suppression ratio (%).

Figure 1

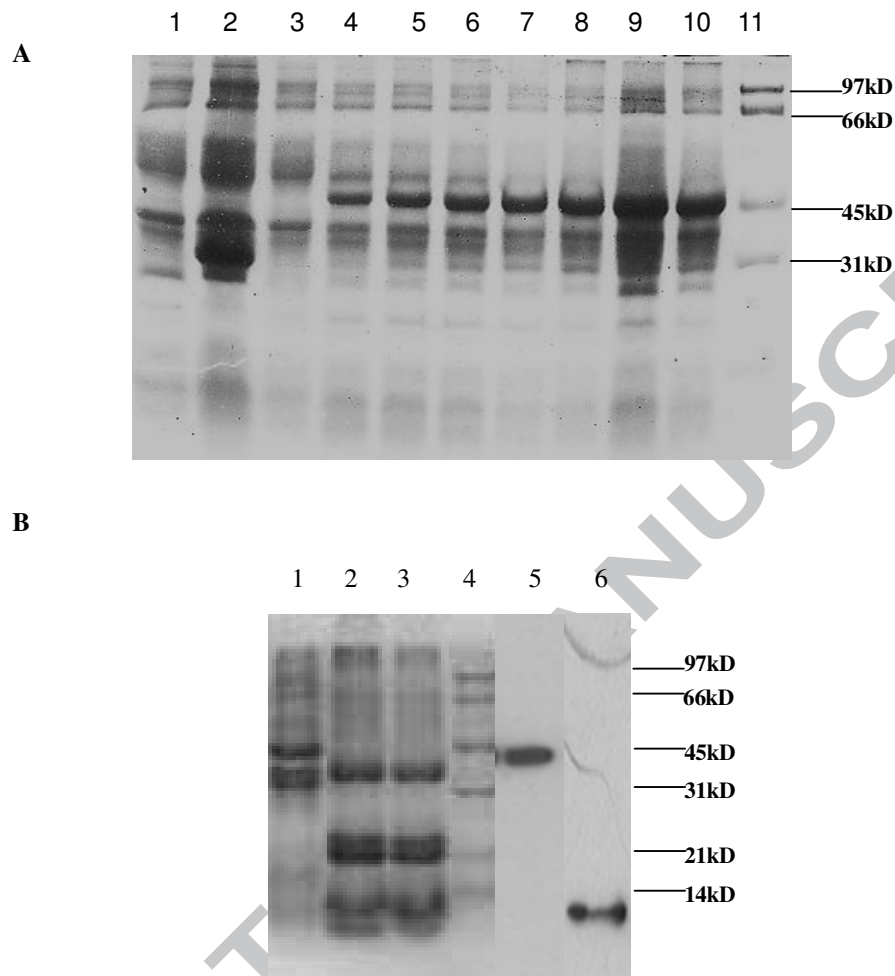
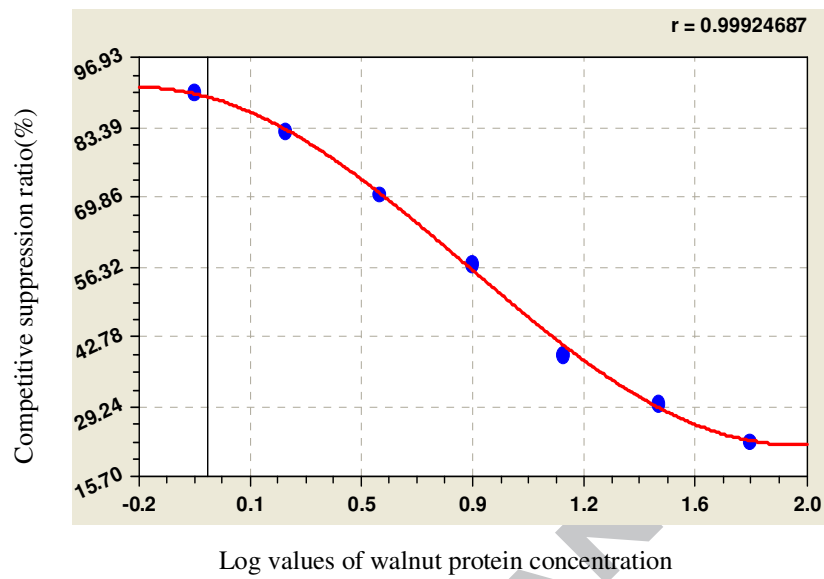


Figure 2

419 **Table 1 The examination of the raw and roasted walnut** The walnut soluble
 420 protein from raw and roasted walnut were examined using the developed ELISA,
 421 the values were obtained after six experiments. The values of the LOQ were not
 422 significantly different($P>0.5$), the LOQ values of raw and roasted walnut were
 423 2.2 and 2.1 $\mu\text{g/g}$ respectively.

424

Sample	The values of the LOQ($\mu\text{g/g}$)						mean value	P value
							($\mu\text{g/g}$)	
Raw walnut	2.3	2.1	2.2	2.4	2.0	2.2	2.2	>0.5
Heated walnut	2.0	2.2	2.1	1.9	2.3	2.1	2.1	

425

Table 2 Cross-Reactivity of various samples using the developed ELISA

The IC₅₀ value of walnut was 1.94 µg/g, the IC₅₀ value of pecan was 985 µg/g, the IC₅₀ values of other plant samples were greatly more than the IC₅₀ value of the walnut with the developed ELISA.

samples	Mean^a IC₅₀ (µg/g)	samples	mean^a IC₅₀ (µg/g)
Walnut	1.94	Hickory	> 1000
Pecan	985	Pistachio nut	> 1000
Cashew	> 1000	Peanut	> 1000
Brazil nut	> 1000	Hazelnut	> 1000
Almond	> 1000	Korean Pine seed	> 1000
Sunflower seeds	> 1000	Sesame	> 1000
Pumpkinseed	> 1000	Rice	> 1000
Corn	> 1000	Soy	> 1000
Common wheat powder	> 1000	Milk	> 1000
Beef	> 1000	Mutton	> 1000
Chicken	> 1000	Pork	> 1000
Duck	> 1000		

- a. Mean shows the average concentration (micrograms of walnut soluble protein weight per gram of food weight) of the walnut soluble proteins.

Table 3 Recovery of walnut protein from matrixes. 4 model samples were prepared, every sample repeated 6 times, the mean recoveries for all ranged from 86 % to 112 %. The coefficient of variation (CV) for the 4 model foods was 6.4 % -8.7 %.

Model food	Mean ($\mu\text{g/g}$) ^a	Recovery (%) ^a	CV (%) ^a
Common wheat powder mixed with walnut protein	11.2	112	8.7
Rice powder mixed with walnut protein	10	100	6.4
Corn powder mixed with walnut protein	10.8	108	7.5
Soy powder mixed with walnut protein	8.6	86	8.1

a. The precision was calculated from six replicates of extraction from the same model processed food. The model foods contained $0\mu\text{g/g}$ of walnut soluble proteins. Mean=concentration of walnut soluble proteins. Recovery = mean/ $10\mu\text{g/g} \times 100$. CV %=coefficient of variation.

Highlights

- A rapid immunoassay system was developed to detect walnut proteins in foods.
- This immunoassay system is specific and relatively sensitive.
- This method is suitable for the quantitative measurement of walnut proteins.